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NEW TREMATODES OF THE SUBFAMILY RENIFERINAE, WITH A DISCUSSION OF THE SYSTEMATICS OF THE GENERA AND SPECIES ASSIGNED TO THE SUBFAMILY GROUP

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The subfamily RENIFERINAE was established by Pratt (1902) for the reception of the genera *Styphlodora* Looss, 1899, *Astiotrema* Looss, 1900, *Renifer* Pratt, 1902, *Ochetosoma* Braun, 1902, and *Oistosomum* Odhner, 1902. Baer (1924) raised the subfamily RENIFERINAE to the rank of family and divided the newly created family RENIFERIDAE into three subfamilies, RENIFERINAE, ENODIOTREMATINAE, and STYPHLOTREMATINAE. Under the subfamily RENIFERINAE Baer included the following genera: *Renifer* Pratt, 1902, *Ochetosoma* Braun, 1902, *Lechriorchis* Stafford, 1905, and *Zeugorchis* Stafford, 1905. Mehra (1931) concluded that Baer's classification was based on insufficient evidence for a natural grouping, but retained the subfamily RENIFERINAE as one of the subgroups of the family PLAGIORCHIIDAE Lühe, 1901 (= LEPODERMATIDAE Odhner, 1910). In the subfamily RENIFERINAE Mehra included eleven genera as follows: *Renifer* Pratt, 1902, *Ochetosoma* Braun, 1902, *Lechriorchis* Stafford, 1905, *Zeugorchis* Stafford, 1905, *Pneumatophilus* Odhner, 1910, *Dasymetra* Nicoll, 1911, *Xenopharynx* Nicoll, 1912, *Mediorima* Nicoll, 1914a, *Dolichopera* Nicoll, 1914b, *Stomatrema* Guberlet, 1928, and *Platymetra* Mehra, 1931. Talbot (1934) rediagnosed the subfamily RENIFERINAE to include the following seven genera: *Macrodera* Looss, 1899 (= *Saphedera* Looss, 1902), *Renifer* Pratt, 1902, *Lechriorchis* Stafford, 1905, *Zeugorchis* Stafford, 1905, *Pneumatophilus* Odhner, 1910, *Dasymetra* Nicoll, 1911, and *Caudorchis* Talbot, 1933. Price (1935, 1936) has considered *Caudorchis* Talbot to be synonymous with *Zeugorchis* Stafford, and has concluded that certain species formerly included in the genus *Zeugorchis* are not cogeneric with *Z. aequatus,* the type species of the genus, and tentatively created a new genus, *Pseudorenifer,* for their reception. McMullen (1937) has rediagnosed the superfamily PLAGIORCHIOIDEA Dollfus, 1930, to include all digenetic trem-

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atodes developing from xiphidiocercariae. Basing his conclusions on evidence obtained from a study of both marital and larval characters of 35 species belonging to 20 genera, 8 subfamilies, and 3 families, McMullen has found it possible to divide the superfamily into six families. The basic plan of the excretory system of both larval and adult forms is used as the main criterion by which the superfamily PLAGIORCHIOIDEA is divided into the families PLAGIORCHIIDAE Lühe, 1901, RENIFERIDAE Baer, 1924, MACRODEROIDIDAE McMullen, 1937, HAPLOMETRIDAE McMullen, 1937, and tentatively LISSORCHIIDAE Magath, 1917, and LECITHODENDRIIDAE Odhner, 1910. More recently Mehra (1937) has reconsidered the classification of the family PLAGIORCHIIDAE, and has placed sixteen genera in the subfamily RENIFERINAE as follows: *Macrodera* Looss, 1899, *Renifer* Pratt, 1902, *Lechriorchis* Stafford, 1905, *Zeugorchis* Stafford, 1905, *Pneumatophilus* Odhner, 1910, *Dasymetra* Nicoll, 1911, *Xenopharynx* Nicoll, 1912, *Dolichopera* Nicoll, 1914b, *Aptorchis* Nicoll, 1914b, *Stomatrema* Guberlet, 1928, *Platymetra* Mehra, 1931, *Pseudorenifer* Price, 1935, *Bilorchis* Mehra, 1937, *Ptyasiorchis* Mehra, 1937, *Ophiorchis,* Mehra, 1937, and *Natriodera* Mehra, 1937.

The writers agree with McMullen (1937) that a close relationship exists between flukes developing from xiphidiocercariae and that both adult and larval characters must be considered as a unit before a natural scheme of classification can be developed. We, therefore, accept the family RENIFERIDAE Baer, 1924, as defined by McMullen.

In the present paper we direct attention to the subfamily RENIFERINAE of the family RENIFERIDAE. Seven new species and two new genera are added to the subfamily group.

Keys for the separation of the genera and species are appended.

SUBFAMILY RENIFERINAE PRATT, 1902

Subfamily diagnosis: RENIFERIDAE. Distomate trematodes with elongated oval bodies and rounded ends, with or without spines. Prepharynx usually present. Esophagus and pharynx usually with gland cells. Ceca variable in length, reaching to or beyond center of body. Ovary behind acetabulum, smooth or lobed in outline. Laurer's canal present. Receptaculum seminis absent. Uterus descending and ascending between testes, descending to posterior end of body. Metraterm present, variable in length and degree of development. Vitellaria follicular, follicles distinct or dentritic, usually lateral to ceca in central third of body except in *Natriodera.* Genital pore in front of acetabulum, median or lateral. Testes smooth to lobate, opposite or oblique, behind ovary. Cirrus sac well developed, rarely extending posterior to ventral sucker. Excretory vesicle Y-shaped, cornua in adults encircling acetabulum except in *Natriodera.* Flame cell pattern of $2\times6\times3$ type. Larval stages remarkably uniform; eggs fully embryonated when oviposited; miracidia with two large penetration glands, without eye-spots; sporocysts simple; xiphidiocercariae with small stylet, 8–10 pairs of penetration glands, and Y-shaped excretory vesicle with cornua encircling acetabulum; adolescercariae using various species of tadpoles as intermediate hosts. Parasitic in Reptilia.

Type genus: Renifer Pratt, 1902.

To the subfamily RENIFERINAE as diagnosed above belong the genera *Renifer* Pratt, 1902, *Lechriorchis* Stafford, 1905, *Zeugorchis* Stafford, 1905, *Pneumatophilus* Odhner, 1910, *Dasymetra* Nicoll, 1912, *Natriodera* Mehra, 1937, *Neorenifer* n. gen., and *Paralechriorchis* n. gen.

In the present diagnosis of the subfamily RENIFERINAE we agree with Talbot (1934) in excluding the genera *Styphlodora* Looss, 1899, *Astiotrema* Looss, 1900, *Ochetosoma* Braun, 1902, and *Oistosomum* Odhner, 1902, for reasons stated by Talbot. Of the genera included in the RENIFERINAE by Mehra (1931) we cannot accept the genera *Xenopharynx* Nicoll, 1912, *Dolichopera* Nicoll, 1914b, *Stomatrema* Guberlet, 1928, and *Platymetra* Mehra, 1931. The genus *Xenopharynx* is excluded since it possesses a well defined receptaculum seminis. The type of uterine pattern, the position of the acetabulum, and the position of the gonads excludes the genus *Dolichopera,* while the presence of a well defined receptaculum seminis and the anteriorly dispersed vitellaria will not permit the inclusion of the genus *Stomatrema.* The genus *Platymetra,* created for the reception of *Styphlodora bascaniensis* Goldberger, 1911, is considered to be synonymous with *Styphlodora* Looss, 1899, and is considered to be the type genus of the subfamily STYPHLODORINAE Dollfus, 1937. Of the genera included in the subfamily RENIFERINAE by Mehra (1937) we cannot accept, in addition to the above mentioned genera, the genera *Aptorchis* Nicoll, 1914, *Bilorchis* Mehra, 1937, *Ptyasiorchis* Mehra, 1937, and *Ophiorchis* Mehra, 1937, *Macrodera* Looss, 1899, and *Pseudorenifer* Price, 1935, being discussed later in this paper. The genus *Aptorchis* shows affinities to the subfamily STYPHLODORINAE and is tentatively placed in that subfamily. The genus *Bilorchis* is more closely related to the members of the subfamily PLAGIORCHIINAE than to the RENIFERINAE due to its organization of body structures, the arrangement of the vitellaria, and the size and shape of the cirrus sac. Price (1938) has pointed out that both *Ophiorchis* and *Ptyasiorchis* are synonymous with *Allopharynx* Strom, 1928, with whom we are in agreement.

In reviewing the taxonomy of the genera belonging to the subfamily RENIFERINAE we are of the opinion the present classification as we find it is at fault due mainly to an oversight in previous interpretation of the diagnosis of the genus *Renifer.* Considering the genus from as critical a standpoint as published descriptions will permit, it appears to us the genus *Renifer* should be defined to include all RENIFERINAE in which the genital pore is situated laterally, outside of the area between the acetabulum and the bifurcation of the ceca, and on the level of the bifurcation of the ceca. Pratt (1903), in his diagnosis of the genus *Renifer,* designated *R. ellipticus* Pratt, 1903, as the type species. When we consider *R. ellipticus,* we find it typical for the subfamily group, but the

position of the genital pore is described and illustrated as being midway between the suckers, close to the margin of the body, at about the level of the bifurcation of the ceca. If we accept the position of the genital pore as being of generic value, we must conclude that *R. ellipticus* cannot be cogeneric with such species as *R. elongatus* Pratt, 1903, *R. kansensis* Crow, 1913, *R. aniarum* Leidy, 1890, etc., in which the genital pore is described as being placed laterally alongside the oral sucker or pharynx. Recently Price (1935, 1936) has given a rediagnosis of the type species of two genera, *Lechriorchis* and *Zeugorchis,* belonging to the subfamily group. In his discussion of the genera Price considered *Caudorchis* Talbot, 1933, to be synonymous with *Zeugorchis* Stafford, 1905. It is further Price's opinion that the species *Z. bosci* (Cobbold, 1859), *Z. ancistrodontis* (MacCallum, 1921), *Z. syntomentera* Sumwalt, 1926, and *Z. megametricus* Talbot, 1934, included in the genus *Zeugorchis* by Talbot (1934), are not cogeneric with *Z. aequatus* Stafford, 1905, the type species. Through a similarity in location of the genital pore and the length of the ceca, Price relates *Z bosci* to the genus *Dasymetra* Nicoll, 1911, and tentatively created a new genus, *Pseudorenifer,* for the reception of the other species discarded from the genus *Zeugorchis.* Price designated *P. megametricus* (Talbot) as the type species for the newly created genus and gave it Talbot's diagnosis for the genus *Zeugorchis.* Among other characters typical for the genus *Zeugorchis* as diagnosed by Talbot, this writer (1934) gave the location of the genital pore as being close behind or to one side of the bifurcation of the ceca. It is our opinion, therefore, that the genus *Zeugorchis* should be characterized mainly on two very prominent features, the position of the genital pore, median and close behind the bifurcation of the ceca, and the location of the testes, in the posterior fourth of the body. The genus *Renifer,* then, should be reserved for those species in which the genital pore is lateral in position and just to one side of the bifurcation of the ceca. Since Price (1935) has designated *P. megametricus* (Talbot) as the type species of the genus *Pseudorenifer,* the transfer of this species to the genus *Renifer* renders the former genus a synonym of the latter.

In the present diagnosis of the genera belonging to the subfamily RENIFERINAE a number of species cannot be assigned to any of the existing genera. These species naturally fall into two groups: first, those species in which the genital pore is lateral in position and located anteriorly to the level of the bifurcation of the ceca; and, second, those species in which the genital pore is median close behind the bifurcation of the ceca, but show certain characters that are incompatible with the species assigned to related genera. In our opinion these two groups are sufficiently distinct from each other and from related genera to warrant the creation of new genera for their reception. For the first group we

propose a new genus, *Neorenifer,* due to their close resemblance to the members of the genus *Renifer,* and for the second group we propose the name *Paralechriorchis,* because of the resemblance of these forms to the genus *Lechriorchis.*

KEY TO THE GENERA OF THE SUBFAMILY RENIFERINAE

1. Genital pore median or slightly to one side of midline, confined to the area between acetabulum and bifurcation of ceca ..... 2.
   Genital pore lateral, outside area between acetabulum and bifurcation of ceca . 7.
2. Ovary close behind acetabulum ..... 3.
   Ovary widely separated from acetabulum ..... *Natriodera* Mehra, 1937.
3. Testes placed at or just posterior to middle of body ..... 4.
   Testes in posterior fourth of body ..... *Zeugorchis* Stafford, 1905.
4. Body elliptical; uterus with few wavy loops ..... 5.
   Body spatulate, widest behind testes; uterus with many loops and coils behind acetabulum ..... *Pneumatophilus* Odhner, 1910.
5. Vitellaria follicular, follicles more or less distinct, mainly lateral to ceca .... 6.
   Vitelline follicles more or less dendritic, lateral, ventral and dorsal to ceca ..... *Dasymetra* Nicoll, 1911.
6. Cirrus sac long, muscular; metraterm one-third to one-half length of cirrus sac ..... *Lechriorchis* Stafford, 1905.
   Cirrus sac medium, muscular; metraterm about equal in length to cirrus sac ..... *Paralechriorchis* n. g.
7. Genital pore lateral on level with bifurcation of ceca ..... *Renifer* Pratt, 1902.
   Genital pore lateral, anterior to bifurcation of ceca, in region of pharynx or oral sucker ..... *Neorenifer* n. g.

Genus *Renifer* Pratt, 1902, emend.

Generic diagnosis: RENIFERINAE. With the characters of the subfamily. Ceca variable in length, may or may not be directed toward center of body between testes. Genital pore lateral outside area between bifurcation of ceca and acetabulum, on level with bifurcation of ceca. Metraterm variable, usually well developed and muscular, usually pulled away from cirrus sac. Cirrus sac muscular, not extending posterior to acetabulum. Parasitic in upper digestive tract of snakes.

Type species: Renifer ellipticus Pratt, 1903.

Additional species: R. ancistrodontis MacCallum, 1921, *R. megametricus* (Talbot, 1934), *R. brachyoesophagidius* (Allison and Holl, 1937), *R. magnus* n. sp., and *R. laterotrema* n. sp.

In the reallocation of species to the genus *Renifer* it has been found necessary to assume the position of the genital pore is of generic value. In so doing we are able to clear up considerably the confusion concerning the key genus, *Renifer,* of the subfamily RENIFERINAE. In an attempt to clear up the taxonomy of the subfamily, Talbot (1934) utilized the degree of development of the metraterm and its relation to the cirrus sac as the main criteria for the separation of the genus *Zeugorchis* from the genus *Renifer,* stating that the main differences between these two genera were the well developed metraterm and its separation from the cirrus sac in *Zeugorchis,* whereas the structure was weakly developed and closely adherent to the cirrus sac in *Renifer.* We cannot accept these characters as being of generic value since they are too variable, the degree of expression depending somewhat on the investigator who handles the worms at the time of preservation. On the other hand the type species of

the genus, *R. ellipticus,* does not show the typical condition described for the group since in this species the metraterm is very slightly pulled away from the cirrus sac. If we consider *Neorenifer heterodontis* n. sp. we find the metraterm to be well developed and pulled away from the cirrus sac, but the genital pore is well forward, at the level of the pharynx.

Price (1935, 1936), in redescribing the type species of the genus *Zeugorchis,* noted that certain species included in the genus were not cogeneric with *Z. aequatus,* consequently he tentatively created a new genus, *Pseudorenifer,* for their reception. These species, with the exception of *Z. aequatus,* were those included in the genus *Zeugorchis* by Talbot (1934). In our opinion these species are cogeneric with *Renifer ellipticus,* the type species of the genus *Renifer,* and are transferred to that genus. Since Price (1935) designated *Zeugorchis megametricus* Talbot, 1934 as the type of the genus *Pseudorenifer,* the transfer of this species to the genus *Renifer* renders *Pseudorenifer* a synonym of the genus *Renifer.*

Of the species formerly included in the genus *Renifer* only two, *R. ellipticus* Pratt, 1903, and *R. ancistrodontis* MacCallum, 1921, are retained, the remainder of the species being considered incompatible with the type species of the genus because of the location of the genital pore. These species are included in a new genus, for which we propose the name *Neorenifer,* and will be discussed under that genus. Likewise certain of the species included in the genera *Zeugorchis* and *Pseudorenifer* are incompatible with any of the existing genera belonging to the subfamily. These species are placed in a new genus, for which we propose the name *Paralechriorchis,* and will be discussed in connection with that genus.

Renifer magnus n. sp.
(Figs. 7 and 11)

Specific diagnosis: Renifer. Body elongated, *8.30 ± .13 (7.70–9.30) mm long by 2.10 ± .01 (2.00–2.30) mm wide. Cuticula beset with spines. Oral sucker wider than long, 0.53 ± .08 (0.50–0.56) mm long by 0.59 ± .09 (0.54–0.62) mm wide. Acetabulum larger, 0.89 ± .10 (0.85–0.94) mm long by 0.96 ± .10 (0.90–1.00) mm wide, situated 2.45 ± .03 (2.10–2.80) mm from anterior margin of body. Prepharynx present. Pharynx muscular, with gland cells, wider than long, 0.28 ± .05 mm in diameter. Esophagus variable, approximately 0.50 mm long. Ceca slender tubes,

* Measurements used in the description of the species *Renifer magnus, R. laterotrema, Neorenifer georgianus, N. drymarchon,* and *N. heterodontis,* are given in two ways: 1. The mean with its probable error. It is exactly an even chance that the true results lie either inside or outside the limits set by the probable error in the plus and minus direction. In computing the probable error of samples in which the number of observations is small, the number of degrees of freedom is taken into account. 2. The smallest to largest measurement in parenthesis. This range is omitted when all measuerments are approximately identical. For the species *Lechriorchis abduscens* and *Neorenifer glandularis* the actual measurements are given since in both cases the total number of observations is too small to permit of statistical treatment.

reaching to posterior margin of testes or to level about 1.00 mm beyond testes, may or may not be clasped between testes. Genital pore ventral, lateral, on level with bifurcation of ceca. Testes large, smooth to irregular in outline, usually oblique, just behind ovary, elongated oval, 1.17 ± .02 (1.00–1.30) mm long by 0.61 ± .07 (0.50–0.70) mm wide. Vasa efferentia uniting on entering cirrus sac. Cirrus sac well developed, large and muscular, 2.45 ± .09 (2.30–2.60) mm long, containing much coiled vesicula seminalis, dilated pars prostatica with gland cells, slender ductus ejaculatorius, and weakly developed cirrus. Excretory system typical for subfamily. Ovary globular, 0.36 ± .01 (0.29–0.43) mm in diameter, close behind ventral sucker, to right of midline. Shell gland and Laurer's canal present. Vitellaria follicular, follicles separated into clusters, nine to twelve clusters on each side of body, from midway between bifurcation and acetabulum to posterior margin of posterior testis. Uterus typical for genus. Metraterm muscular, about one-third length of cirrus sac. Ova numerous, operculated, 45–50 μ by 26–30 μ.

Host: Drymarchon corais couperi (Holbrook).
Habitat: Esophagus.
Locality: Texas, U. S. A. (Zoological Park, New Orleans, Louisiana).
Type specimen: U. S. Nat. Mus. Helm. Coll. No. 9131.

Renifer magnus is described from five specimens taken from the esophagus of an indigo snake, *Drymarchon corais couperi,* that died in the New Orleans Zoological Park shortly after it had been shipped there from southwest Texas. The species appears to be distinct from the other members of the genus due to its unusually large size. The large size of the testes, the enormous development of the metraterm and cirrus sac, and the distribution and clustered arrangement of the vitellaria serve to separate it from all other species now included in the genus *Renifer.*

Renifer laterotrema n. sp.
(Fig. 12)

Specific diagnosis: Renifer. Body small, with almost evenly rounded ends and parallel sides, 3.20 ± .07 (2.00–4.30) mm long by 1.10 ± .02 (0.80–1.40) mm wide. Cuticula covered with spines. Oral sucker 0.43 ± .02 (0.34–0.52) mm in diameter. Acetabulum smaller, 0.38 ± .04 (0.35–0.50) mm in diameter, placed 1.20 ± .05 mm behind anterior end of body. Prepharynx present. Pharynx muscular, with gland cells, approximately 0.18 mm in diameter. Esophagus about 0.25 mm long, with gland cells. Ceca tubular, extending to approximately 0.90 mm beyond testes, with ends directed toward center of body. Genital pore marginal, on level with bifurcation of ceca or slightly caudal to that level. Testes small, opposite or slightly oblique, close behind ovary, smooth to irregular in outline, 0.24 ± .03 (0.18–0.36) mm long by 0.17 ± .02 (0.11–0.24) mm wide. Vasa efferentia uniting on entering cirrus sac. Cirrus sac muscular, medium in size, approximately 0.80 mm long, often lying almost transversely across left half of body with distal end resting on anterior margin of ventral sucker, or curving around ventral sucker to end near midline of sucker on opposite side from genital pore, containing elongated and much coiled vesicula seminalis, almost globular pars prostatica with gland cells, short, slender ductus ejaculatorius, and weakly developed cirrus. Excretory system typical for subfamily. Ovary close behind or just to one side of posterior boundary of acetabulum, right of midline, globular, 0.17 ± .03 (0.14–0.22) mm in diameter. Shell gland and Laurer's canal present. Vitellaria follicular, mainly lateral to ceca, from middle of acetabulum to near ends of ceca. Uterus almost straight tube with slight spiral-like coiling, much dilated posteriorly. Metraterm muscular, about one-third length of cirrus sac, slightly separated from cirrus sac. Ova numerous, operculated, 48 μ by 20–24 μ.

Host: Agkistrodon piscivorus (Lacépède).
Habitat: Esophagus.
Locality: Raceland, Louisiana, U. S. A.
Type specimen: U. S. Nat. Mus. Helm. Coll. No. 9132.

Renifer laterotrema is described from nine specimens taken from the esophagus of the cotton-mouth moccasin, *Agkistrodon piscivorus,* from Raceland, Louisiana. The position of the genital pore at the margin of the body on level with the bifurcation of the caeca, the transverse position of the cirrus sac, the size of the acetabulum in relation to the size of the oral sucker, and the more caudal distribution of the vitellaria distinguishes the species from the other members of the genus *Renifer.*

KEY TO THE SPECIES OF THE GENUS *Renifer*

1. Intestinal ceca long, extending to or beyond testes ..... 2.
   Intestinal ceca short, ending in front of testes ..... 3.
2. Genital pore on margin of body; testes small ..... *R. laterotrema* n. sp.
   Genital pore not on margin of body; testes large ..... *R. magnus* n. sp.
3. Esophagus long (more than 0.10 mm in length) ..... 4.
   Esophagus short ..... *R. brachyoesophagidius* (Allison & Holl, 1937).
4. Vitellaria extending to posterior margin of testes ..... 5.
   Vitellaria anterior to testes ..... *R. megametricus* (Talbot, 1934).
5. Metraterm widely separated from cirrus sac. *R. ancistrodontis* MacCallum, 1921.
   Metraterm not widely separated from cirrus sac .... *R. ellipticus* Pratt, 1903.

Genus *Lechriorchis* Stafford, 1905

Generic diagnosis: RENIFERINAE. With the characters of the subfamily. Acetabulum larger than oral sucker. Ends of ceca usually directed toward midline of body. Ovary close behind acetabulum. Metraterm well developed, muscular, about one-third length of cirrus sac. Cirrus sac large, muscular, not extending posterior to acetabulum. Genital pore median or slightly displaced to right or left of midline, between bifurcation of ceca and anterior boundary of acetabulum. Testes close behind ovary. Parasitic in respiratory, digestive, and reproductive tracts of snakes.

Type species: Lechriorchis primus Stafford, 1905.

Additional species: L. megasorchis (Crow, 1913), *L. propria* (Nicoll, 1914a), *L. plesientera* Sumwalt, 1926, *L. tygarti* Talbot, 1933, and *L. abduscens* n. sp.

For a discussion of the genus and its assigned species one is referred to Talbot (1934), who has given reasons for synonymies, and with whom we are in agreement. The species *L. secundus* Canavan, 1937, appears to belong to the genus *Paralechriorchis* n. g. and will be discussed in connection with that genus.

Lechriorchis abduscens n. sp.
(Fig. 13)

Specific diagnosis: Lechriorchis. Body much elongated, broadly rounded anteriorly, more pointed posteriorly, 7.60 mm long by 1.75 mm wide, widest at level of acetabulum. Cuticula with spines. Oral sucker 0.56 mm long by 0.60 mm wide. Acetabulum 0.84 mm in diameter, placed 2.30 mm from anterior margin of body. Prepharynx short. Pharynx muscular, 0.22 mm in diameter, with gland cells. Esophagus short, 0.16 mm long, slightly muscular, with gland cells. Ceca long, reaching a short distance beyond testes, ends dilated, may or may not be clasped between testes. Genital pore displaced to left of midline, close behind bifurcation of

ceca. Testes smooth to irregular in outline alternating in the oblique position, one testis more advanced than the other by half its long diameter, 1.26 mm long by 0.44 mm wide, placed about 0.60 mm behind ovary. Vasa efferentia uniting on entering cirrus sac. Cirrus sac large, 2.12 mm long, extending from middle of acetabulum through a considerable loop to genital pore, containing much coiled vesicula seminalis, almost spherical pars prostatica with gland cells, long, slender ductus ejaculatorius, and slightly muscular cirrus. Excretory system typical for the subfamily. Ovary spherical, 0.24 mm in diameter, right of midline close behind acetabulum. Shell gland and Laurer's canal present. Vitellaria lateral and ventral to ceca, follicles form about eleven distinct clusters on each side of body, from midway between acetabulum and genital pore to middle of anterior testis. Uterus typical for genus. Metraterm well developed, muscular, about one-third length of cirrus sac. Ova numerous, operculated, 45 μ by 27 μ.

Host: *Lampropeltis getulus holbrooki* (Stejneger).
Habitat: Lung.
Locality: Raceland, Louisiana, U. S. A.
Type species: U. S. Nat. Mus. Helm. Coll. No. 9133.

Lechriorchis abduscens is described from two fully matured specimens taken from the membranous sack-like posterior portion of the lung of a king snake, *Lampropeltis getulus holbrooki,* from Raceland, Louisiana. The two specimens are so near the same size in every detail that it is difficult to distinguish one specimen from the other. The species appears to be more closely related to *L. tygarti* than to the other members of the genus. From this species *L. abduscens* can be distinguished by its larger body, the length and development of the cirrus sac, the slightly displaced genital pore, the larger testes, and the grouped follicles of the vitellaria. The present species resembles *L. megasorchis* in regard to the large size of the testes, but when the other features of the body are considered these two species are quite distinct.

KEY TO THE SPECIES OF THE GENUS *Lechriorchis*

1. Intestinal ceca ending in advance of testes ..... 2.
   Intestinal ceca extending beyond testes ..... 3.
2. Acetabulum in first body third ..... *L. propria* (Nicoll, 1914a).
   Acetabulum in second body third ..... *L. tygarti* Talbot, 1933.
3. Body small, less than 7.00 mm long; testes less than 1.00 mm. long; cirrus sac less than 2.00 mm long ..... 4.
   Body large; testes large; cirrus sac long ..... *L. abduscens* n. sp.
4. Acetabulum considerably larger than oral sucker ..... 5.
   Suckers about the same size ..... *L. megasorchis* (Crow, 1913).
5. Acetabulum in first body third ..... *L. primus* Stafford, 1905.
   Acetabulum in central body third ..... *L. plesientera* Sumwalt, 1926.

Genus *Zeugorchis* Stafford, 1905

Generic diagnosis: RENIFERINAE. With the characters of the subfamily. Genital pore median or to one side of midline, close behind bifurcation of ceca. Ovary median or to one side of midline, close behind caudal boundary of acetabulum. Metraterm muscular, about one-half length of cirrus sac. Testes opposite, in posterior fourth of body, close behind ends of ceca. Cirrus sac muscular, may or may not extend posterior to acetabulum. Parasitic in digestive and respiratory tracts of snakes.

Type species: *Zeugorchis aequatus* Stafford, 1905.
Additional species: *Z. eurinus* (Talbot, 1933).

Price (1935, 1936) considered *Caudorchis* Talbot, 1933 to be synonymous with *Zeugorchis,* and has transferred *C. eurinus* Talbot, the type and only species of the genus to the genus *Zeugorchis.* We are in agreement with this transfer of species.

KEY TO THE SPECIES OF THE GENUS *Zeugorchis*

1. Cirrus sac long; ovary large; vitellaria occupying more than central third of body ..... *Z. aequatus* Stafford, 1905.
   Cirrus sac short; ovary small; vitellaria confined to central third of body. *Z. eurinus* (Talbot, 1933).

Genus *Pneumatophilus* Odhner, 1910

Generic diagnosis: RENIFERINAE. With the characters of the subfamily. Body more or less spatulate, with anterior end somewhat attenuated and caudal end broadly rounded. Acetabulum larger than oral sucker. Ceca usually with lateral pouchings. Genital pore median, close behind bifurcation of ceca. Ovary close behind acetabulum. Uterus greatly convoluted, thrown into series of vertical and transverse loops in region posterior to acetabulum. Metraterm muscular, about two-thirds length of cirrus sac. Testes opposite, not greatly separated from ovary, smooth or deeply notched in outline. Cirrus sac moderate in size, not extending posterior to acetabulum. Parasitic in respiratory tract of snakes.

Type species: Pneumatophilus variabilis (Leidy, 1856).

Additional species: P. foliaformis Talbot, 1934, and *P. leidyi* Byrd & Denton, 1937.

KEY TO THE SPECIES OF THE GENUS *Pneumatophilus*

1. Testes notched to lobed in outline ..... 2.
   Testes smooth in outline ..... *P. foliaformis* Talbot, 1934.
2. Testes wider than long; ceca ending at anterior margin of testes. *P. leidyi* Byrd & Denton, 1937.
   Testes as broad as long; ceca extending to posterior margin of testes. *P. variabilis* (Leidy, 1856).

Genus *Dasymetra* Nicoll, 1911

Generic diagnosis: RENIFERINAE. With the characters of the subfamily. Body rather thick and muscular, with rounded ends. Esophagus short, broad, muscular, with gland cells. Ceca wide, extending to near posterior end of body, lined internally with rather tall epithelium. Genital pore slightly to one side of midline, at or close behind bifurcation of ceca. Ovary close behind acetabulum. Uterus forming characteristic knot-like loop at posterior end of body before expanding to form almost straight ascending limb. Metraterm very muscular, about one-half length of cirrus sac. Vitellaria dendritic, ventral, lateral, and dorsal to ceca, sometimes forming dorsal bridge in region of ovary. Cirrus sac large, not extending posterior to acetabulum. Parasitic in upper digestive tract of snakes.

Type species: Dasymetra conferta Nicoll, 1911.

Additional species: D. villicaeca Byrd, 1935, and *D. nicolli* Holl and Allison, 1935.*

KEY TO THE SPECIES OF THE GENUS *Dasymetra*

1. Testes smooth in outline ..... 2.
   Testes deeply notched laterally ..... *D. nicolli* Holl & Allison, 1935.
2. Vitelline follicles separated into lateral fields ..... *D. conferta* Nicoll, 1911.
   Vitelline follicles becoming confluent from side to side in region dorsal to ovary. *D. villicaeca* Byrd, 1935.

* Footnote added in proof: The species *Zeugorchis longicirrus,* recently described by Odlaug (1938, Tr. Am. Micr. Soc. **57**: 173–177), appears to belong to the genus *Dasymetra* Nicoll rather than to the genus *Zeugorchis* Stafford due to

Genus *Natriodera* Mehra, 1937

Generic diagnosis: RENIFERINAE. With the characters of the subfamily. Body greatly elongated and slender. Esophagus long. Ovary in middle of body, widely separated from acetabulum. Vitellaria lateral, posterior to level of ovary. Uterus with transverse coils in middle of body and large vertical loops filling body posterior to testes. Metraterm weakly developed. Genital pore close in front of acetabulum, to one side of midline. Testes widely separated from ovary. Cirrus sac long and slender, reaching behind acetabulum. Parasitic in lungs of snakes.

Type and only species: Natriodera verlatum (Talbot, 1934).

We are in agreement with Mehra (1937) in considering *Macrodera verlatum* Talbot, 1934 to represent a form not cogeneric with *Macrodera longicollis* (Abildgaard, 1788). For the species *Macrodera verlatum* Talbot, Mehra has proposed a new genus *Natriodera* with *N. verlatum* (Talbot) as the type species. Talbot (1934) has included the genus *Macrodera* Looss, 1899, in the subfamily RENIFERINAE, removing it from the subfamily SAPHEDRATINAE Baer, 1924. Mehra (1937) has accepted this transfer of the genus *Macrodera* and has created the subfamily PNEUMONOECESINAE for the reception of the other genera included in the SAPHEDRATINAE. These changes are not acceptable in the light of the recent paper by McMullen (1937) who has shown a close relationship to exist between the subfamilies HAPLOMETRINAE Pratt, 1902, including the genera *Haplometra, Haematoloechus* and *Ostiolum,* and the PROSTHOGONIMINAE Lühe, 1909, including *Prosthogonimus* and related genera. For these two subfamilies McMullen has created the family HAPLOMETRIDAE. We are of the opinion the genus *Macrodera* should be included in the subfamily HAPLOMETRINAE because of a close resemblance between it and the members of the genus *Haematoloechus.* The subfamily PNEUMONOECESINAE Mehra is, therefore, considered as a synonym of the subfamily HAPLOMETRINAE Pratt. It is further suggested that the species of *Macrodera* reported by Mödlinger (1924) is not cospecific with *M. longicollis.*

Genus *Neorenifer* n. g.

Generic diagnosis: RENIFERINAE. With the characters of the subfamily. Ventral sucker larger than oral sucker. Ends of ceca may or may not be directed toward center of body at ends. Genital pore to one side of midline, on level with pharynx or oral sucker. Ovary close behind acetabulum. Metraterm usually weakly developed, may or may not be closely adherent to cirrus sac. Vitellaria may be divided into two groups in lateral fields, usually as continuous band of glands in central body third. Testes close behind ovary. Cirrus sac usually weakly muscular, not extending posterior to acetabulum. Parasitic in digestive, respiratory, and reproductive tracts of snakes.

Type species: Neorenifer orula (Talbot, 1934).

Additional species: N. sauromates (Poirier, 1885), *N. aniarum* (Leidy, 1890), *N. zschokkei* (Volz, 1899), *N. elongatus* (Pratt, 1903), *N. validus* (Nicoll, 1911),

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the shape of the body, the position of the testes, the muscular metraterm, and the dendritic arrangement of the vitellaria. We, therefore, transfer the species to the genus *Dasymetra* and it becomes *D. longicirrus* (Odlaug).

N. formosum (Nicoll, 1911), *N. kansensis* (Crow, 1913), *N. acetabularis* (Crow, 1913), *N. septicus* (MacCallum, 1921), *N. wardi* (Byrd, 1936), *N. glandularis* n. sp., *N. drymarchon* n. sp., *N. georgianus* n. sp., and *N. heterodontis* n. sp.

The genus *Neorenifer* is closely related to the genus *Renifer,* from which it is separated by two rather prominent characters: 1, the forward location of the genital pore, located to one side of the midline on level with the pharynx or oral sucker, and 2, the rather weakly developed cirrus sac. In the majority of the species assigned to the genus *Neorenifer* the metraterm shows a tendency to be closely adherent to the cirrus sac, although this is not a constant feature. In regards to the other features of the members of the genus there is a marked resemblance to the genus *Renifer.*

From the above list of species included in the genus *Neorenifer* a number of forms fail to appear. In the following paragraphs we propose to show that a number of these are synonyms of earlier species.

We consider *Renifer natricis* MacCallum, 1921, and *R. texanus* Harwood, 1932, to be identical with *R. aniarum* Leidy, 1890, since in practically every detail these two species vary from Leidy's species only a few microns in body size and internal anatomy. These variations are accountable to species variation and are not specific. The only feature by which *R. natricis* may be separated from *R. aniarum* is the size of the ova, which for the former species is said to have ova measuring 50 μ by 20 μ, while the ova for *R. aniarum* vary from 32–45 μ by 20–25 μ. On the other hand the type of uterus exhibited by *R. texanus* serves as the only feature by which it can be separated from *R. aniarum.* When *R. aniarum* is studied in the living condition this difference between the two species is explained, since in the latter species the uterus is greatly distended and thrown into a more convoluted condition before the worm has had time to oviposit the majority of its ova. We consider these two species to be synonymous with *R. aniarum,* and the species becomes *Neorenifer aniarum* (Leidy, 1890) since the genital pore is located to one side of the midline in the region of the oral sucker.

Talbot (1934) has recognized the fact that *Lechriorchis validus* Nicoll, 1911, is cogeneric with the greater majority of the species included in the genus *Renifer,* and has transferred the species to that genus. In a like manner the same author has transferred *Lechriorchis inermis* Lebour, 1913, to the genus *Renifer.* We consider *L. inermis* to be synonymous with *L. validus* since these two species differ only in respect to the absence of spines in the former species. In the description of *L. inermis* we find spines were observed on the integument of certain specimens, but these failed to reach the surface. We agree with Talbot in considering the two species to be related to such a species as *Neorenifer aniarum* rather than to the *Lechriorchis,* but go one step further and

consider *L. inermis* to be synonymous with *L. validus*. The species, then, becomes *Neorenifer validus* (Nicoll, 1911).

Renifer ophiboli MacCallum, 1921, differs from *R. septicus* MacCallum, 1921, only in the more spent condition of the vitellaria, the more shrunken testes and ovary, and the failure of the cirrus sac to enclose the vesicula seminalis. In consideration of the first two differences we are of the opinion they can be explained on the basis of age variation, especially when we are mindful of the condition of these organs in the older specimens of such species as *Paralechriorchis* (=*Zeugorchis*) *syntomentera* (Sumwalt, 1926), *Lechriorchis plesientera* Sumwalt, 1926, and *Neorenifer glandularis* n. sp. In regard to the last difference between these two species it has been our experience that the musculature of the cirrus sac more readily loses its stain than do certain other structures of the organ, especially those containing spermatozoa. On the other hand this condition is not met with in any other member of the RENIFERINAE. When we take into account the full development of the internal anatomy of *R. septicus* and the senescent condition exhibited by *R. ophiboli* we feel justified in considering the two species to be identical. The name *septicus* is selected because of page priority. The species, then, becomes *Neorenifer septicus* (MacCallum, 1921).

Neorenifer georgianus n. sp.
(Fig. 14)

Specific diagnosis: Neorenifer. Body elliptical, bluntly rounded at ends, 3.45 ± .08 (2.92–4.00) mm long by 1.24 ± .018 (1.10–1.40) mm wide. Cuticula beset with spines to testes. Oral sucker 0.34 ± .02 (0.32–0.38) mm in diameter. Acetabulum 0.43 ± .02 (0.34–0.47) mm in diameter, 1.32 ± .06 mm from anterior margin of body. Prepharynx short. Pharynx 0.15 ± .005 mm in diameter, with gland cells. Esophagus 0.18 ± .015 mm long, with gland cells. Ceca reaching to testes, ending in bag-like dilations. Ovary spherical to transversely oval, 0.18 ± .07 (0.14–0.20) mm in diameter, close behind acetabulum and slightly to right of midline. Uterus typical for genus. Metraterm short, weakly developed. Ova numerous, operculated, 42–45 μ by 21–27 μ. Vitellaria follicular, lateral and ventral to ceca, from bifurcation of ceca to 0.30 mm behind testes. Shell gland, small yolk reservoir, and Laurer's canal present. Genital pore near left lateral margin on level with caudal boundary of pharynx. Testes smooth to irregular in outline, opposite to slightly oblique, 0.31 ± .04 mm behind ovary; left testis 0.41 ± .04 (0.34–0.43) mm long by 0.29 ± .04 (0.16–0.47) mm wide; right testis 0.36 ± .03 (0.20–0.50) mm in diameter. Vasa efferentia uniting on entering cirrus sac. Cirrus sac in front of acetabulum, containing much coiled vesicula seminalis, club-shaped pars prostatica with gland cells, long, slender ductus ejaculatorius, and weakly developed cirrus. Excretory system typical for subfamily.

Host: Coluber constrictor constrictor L.
Habitat: Mouth cavity and esophagus.
Locality: Athens, Georgia, U. S. A.
Type species: U. S. Nat. Mus. Helm. Coll. No. 9134.

Neorenifer georgianus is described from nine fully matured specimens from the mouth cavity and esophagus of the black snake, *Coluber constrictor constrictor* L. taken at Athens, Georgia. The species appears

to be more closely related to *N. kansensis* than to the other members of the genus. From this species it is distinguished by its smaller body size, smaller suckers, smaller pharynx, shorter ceca, larger testes, smaller ovary, more posterior genital pore, and more extensive vitellaria.

Neorenifer glandularis n. sp.
(Fig. 15)

Specific diagnosis: Neorenifer. Body elongated oval, bluntly rounded ends, 2.30 mm in length by 1.00 mm wide at level of acetabulum. Cuticula with spines to testes. Conspicuous tube-like integumentary glands forming rows on either side of midline to near posterior end; glands congregating about oral sucker where they become longer and form a distinct double row. Oral sucker 0.33 mm in diameter. Acetabulum 0.46 mm long by 0.43 mm wide, 1.13 mm from anterior margin of body. Prepharynx present. Pharynx 0.13 mm in diameter, with gland cells. Esophagus 0.32 mm long, with gland cells. Ceca large, bag-like, reaching to level of ovary. Ovary 0.13 mm in diameter, close behind acetabulum, to right of midline. Uterus typical, considerably convoluted, descending to near caudal end of body. Metraterm weakly developed. Ova numerous, operculated, 45 μ by 21 μ. Vitellaria follicular, compact, lateral and ventral to ceca, extending from level of acetabulum to posterior border of testes. Genital pore near left lateral margin of body at level of caudal boundary of oral sucker. Testes smooth in outline, opposite, about 1.20 mm behind ovary, 0.25 mm long by 0.22 mm wide. Vasa efferentia uniting on entering cirrus sac. Cirrus sac long, from middle of acetabulum to genital pore, containing small vesicula seminalis, large pars prostatica with numerous gland cells, long slender ductus ejaculatorius, and weakly developed cirrus. Excretory system typical for subfamily.

Host: Sistrurus miliarius barbouri Gloyd.
Habitat: Mouth cavity and esophagus.
Locality: Silver Springs, Florida, U. S. A.
Type specimen: U. S. Nat. Mus. Helm. Coll. No. 9135.

Neorenifer glandularis is described from a single mature specimen, one senescent specimen, and three immature specimens taken from the mouth cavity and esophagus of the ground rattler, *Sistrurus miliarius barbouri* Gloyd, from Silver Springs, Florida. The species appears to be more closely related to *N. kansensis* and *N. septicus* than to the other members of the genus. From *N. kansensis* it is separated by its smaller body size, the presence of dermal glands, smaller pharynx, larger ceca, smaller ovary and testes, and the more posterior distribution of the vitellaria. From *N. septicus* the species is distinguished by its broader body, the presence of dermal glands, more muscular esophagus with gland cells, the smaller ovary and testes, the more posterior distribution of the vitellaria, and the more coiled uterus.

In contrasting the mature specimen with the senescent one it is worth noting that the body of the older specimen is larger due to the greatly distended uterus, the uterus more convoluted, the testes much smaller and more wrinkled, the ovary irregular in outline and smaller, the suckers about one-third larger, and the vitellaria so nearly exhausted that they are scarcely discernible.

Neorenifer drymarchon n. sp.
(Fig. 16)

Specific diagnosis: Neorenifer. Body elongated with almost parallel sides and rounded ends, 6.80 ± .20 (6.00–7.40) mm long by 1.60 ± .04 (1.40–1.75) mm wide, widest in anterior half of body. Cuticula with spines to caudal end of body. Oral sucker 0.47 ± .01 (0.38–0.52) mm in diameter. Acetabulum 0.67 ± .015 (0.45–0.74) mm in diameter, 1.80 ± .03 mm from anterior end of body. Prepharynx short. Pharynx 0.19 ± .10 (0.18–0.20) mm long by 0.26 ± .08 (0.25–0.29) mm wide, with gland cells. Esophagus 0.32 ± .02 mm long, with few gland cells. Ceca slender tubes, reaching to or just beyond anterior margin of testes, with ends turned in toward midline of body. Ovary 0.26 ± .02 (0.25–0.29) mm in diameter, just to right of midline, close behind acetabulum. Uterus with slender, slightly coiled descending limb and much pouched ascending limb, distinctive. Metraterm muscular, one-third length of cirrus sac. Ova numerous, operculated, 34–36 μ by 21 μ. Vitellaria follicular, lateral and ventral to ceca, from anterior margin of acetabulum to level approximately 0.50 mm beyond testes. Genital pore near left lateral margin of body at level of prepharynx. Testes opposite or slightly oblique, slightly irregular to deeply notched in outline, 0.68 ± .15 mm behind ovary, 0.56 ± .01 (0.50–0.60) mm long by 0.31 ± .03 (0.27–0.40) mm wide. Vasa efferentia uniting on entering cirrus sac. Cirrus sac long, from anterior margin of acetabulum to genital pore, typical. Excretory system typical for the subfamily.

Host: Drymarchon corais couperi (Holbrook).
Habitat: Esophagus.
Locality: Texas, U. S. A. (Zoological Park, New Orleans, Louisiana).
Type species: U. S. Nat. Mus. Helm. Coll. No. 9136.

Neorenifer drymarchon is described from four fully matured specimens taken from the esophagus of an indigo snake, *Drymarchon corais couperi* (Holbrook), that died in the New Orleans Zoological Park shortly after being shipped from southwest Texas. The species can be distinguished from the other members of the genus by its large body size, its well developed metraterm, and the pattern made by the uterus.

Neorenifer heterodontis n. sp.
(Figs. 9 and 17)

Specific diagnosis: Neorenifer. Body ovate, 3.80 ± .09 (3.60–4.00) mm long by 1.50 ± .03 (1.50–1.70) mm wide in region of acetabulum. Cuticula with spines to posterior end of body. Inconspicuous dermal glands around oral sucker. Oral sucker 0.45 ± .04 (0.43–0.45) mm in diameter. Acetabulum 0.65 ± .05 (0.61–0.79) mm in diameter, 1.20 ± .02 mm from anterior margin of body. Prepharynx short. Pharynx 0.21 ± .02 (0.20–0.25) mm in diameter, with few rather large gland cells. Esophagus approximately 0.16 mm long. Ceca slender tubes with few dilations, reaching to level approximately 0.50 mm beyond testes. Ovary close behind or dorsal to posterior half of acetabulum, to right of midline, 0.21 ± .02 (0.16–0.23) mm in diameter. Uterus typical for genus. Metraterm well developed, widely separated from cirrus sac, about one-half length of cirrus sac. Ova numerous, operculated, 36–42 μ by 19–21 μ. Vitellaria follicular, follicles grouped into about six to seven clusters on each side, lateral and ventral to ceca, from midway between acetabulum and bifurcation of ceca to level about midway between testes and ends of ceca. Genital pore to left of midline, close beside pharynx, on level with anterior margin of pharynx. Testes approximately opposite, smooth in outline, about their own diameter behind ovary, 0.44 ± .02 (0.27–0.60) mm in diameter. Vasa efferentia uniting on entering cirrus sac. Cirrus sac large, from middle of acetabulum to genital pore, containing much coiled vesicula seminalis, long, flask-shaped pars prostatica with gland cells, rather short ductus ejaculatorius, and short but muscular cirrus. Excretory system typical for subfamily.

Host: Heterodon contortrix (L.).
Habitat: Mouth cavity and esophagus.
Locality: State College, Mississippi, U. S. A.
Type species: U. S. Nat. Mus. Helm. Coll. No. 9137.

Neorenifer heterodontis is described from measurements of ten fully matured specimens mounted from a lot of about fifty specimens collected from the mouth cavity and esophagus of a single specimen of the hog-nosed snake, *Heterodon contortrix* (L.), from State College, Mississippi. The species appears to be distinct from other members of the genus through its ovate body, a remarkable uniformity of internal organs, the well developed and muscular metraterm, the grouped condition of the vitellaria, and the position of the genital pore.

KEY TO THE SPECIES OF THE GENUS *Neorenifer*

1. Vitellaria divided into two groups by the acetabulum ..... 2.
   Vitellaria not divided into two groups as above ..... 4.
2. Esophagus present; oral sucker more than one-half as large as the acetabulum 3.
   Esophagus absent; oral sucker about one-half the size of the acetabulum. *N. acetabularis* (Crow, 1913).
3. Body large, more than 1.80 mm long ..... *N. aniarum* (Leidy, 1890).
   Body small, less than 1.80 mm long ..... *N. wardi* (Byrd, 1936).
4. Ceca long, reaching beyond testes ..... 5.
   Ceca short, ending at or just in front of the testes ..... 8.
5. Genital pore close beside the pharynx, near midline ..... 6.
   Genital pore near lateral margin, between pharynx and margin of body ..... 7.
6. Metraterm short; vitellaria anterior to testes ..... *N. elongatus* (Pratt, 1903).
   Metraterm long; vitellaria extending beyond testes ..... *N. heterodontis* n. sp.
7. Ceca extending to posterior tip of body ..... *N. sauromates* (Poirier, 1885).
   Ceca ending just posterior to testes ..... *N. validus* (Nicoll, 1911).
8. Uterus with few wavy coils in body posterior to acetabulum ..... 9.
   Uterus with many coils or pouches in post-acetabular region ..... 14.
9. Integument of body simple or with few dermal glands ..... 10.
   Integument of body with definite rows of dermal glands on each side of midline and about oral sucker ..... *N. glandularis* n. sp.
10. Acetabulum less than one-third larger than oral sucker; testes round or elongated oval ..... 11.
    Acetabulum more than one-third larger than oral sucker; testes transversely oval ..... *N. zschokkei* (Volz, 1899).
11. Vitellaria extending only to level of testes ..... 12.
    Vitellaria extending beyond testes ..... *N. georgianus* n. sp.
12. Genital pore on right side of body near margin ..... 13.
    Genital pore on left side of body near margin ..... *N. orula* (Talbot, 1934).
13. Testes more than 0.30 mm in diameter ..... *N. septicus* (MacCallum, 1921).
    Testes less than 0.30 mm in diameter ..... *N. kansensis* (Crow, 1913).
14. Uterus with many transverse loops and coils in body posterior to acetabulum. *N. formosum* (Nicoll, 1911).
    Uterus with bag-like pouches on either side of an otherwise straight tube. *N. drymarchon* n. sp.

Genus *Paralechriorchis* n. g.

Generic diagnosis: RENIFERINAE. With the characters of the subfamily. Genital pore median or slightly to one side of midline, at or just posterior to bifurcation of ceca. Metraterm well developed, muscular, approximately as long as cirrus sac. Cirrus sac short, extending from acetabulum to genital pore. Parasitic in digestive and reproductive tracts of snakes.

Type species: Paralechriorchis syntomentera (Sumwalt, 1926).
Additional species: P. bosci (Cobbold, 1859) and *P. natricis* (Holl and Allison, 1935).

The genus *Paralechriorchis* is closely related to no less than three of the genera of the subfamily RENIFERINAE, *viz., Lechriorchis, Zeugorchis*, and *Pneumatophilus*. We are of the opinion the species assigned to the genus *Paralechriorchis* are not cogeneric with the type species of these closely related genera, but possess characteristics quite distinct and should be included in a genus set apart for their reception, for which we propose the name *Paralechriorchis*. Two morphological features serve to distinguish the species of the new genus from closely related genera. 1. The presence of a short, stout cirrus sac that extends the short distance from the acetabulum to the genital pore. 2. The presence of a well developed and muscular metraterm that equals the cirrus sac in length. Neither of these two features are to be found in any of the existing genera of the subfamily.

We find it impossible to distinguish between *Zeugorchis natricis* Holl and Allison, 1935, and *Lechriorchis secundus* Canavan, 1937. Due to this striking similarity of body plan and almost identical size range for the various internal anatomy we feel justified in considering *L. secundus* as a synonym of *Z. natricis*. Since *Z. natricis* possesses the characters of the genus *Paralechriorchis* we transfer it to that genus. The species becomes *Paralechriorchis natricis* (Holl and Allison, 1935).

KEY TO THE SPECIES OF THE GENUS *Paralechriorchis*

1. Ceca short, reaching only to testes . . . . . 2.
   Ceca long, reaching to posterior end of body ..... *P. bosci* (Cobbold, 1859).
2. Ceca ending short of testes ..... *P. syntomentera* (Sumwalt, 1926).
   Ceca reaching to or just beyond testes ..... *P. natricis* (Holl & Allison, 1935).

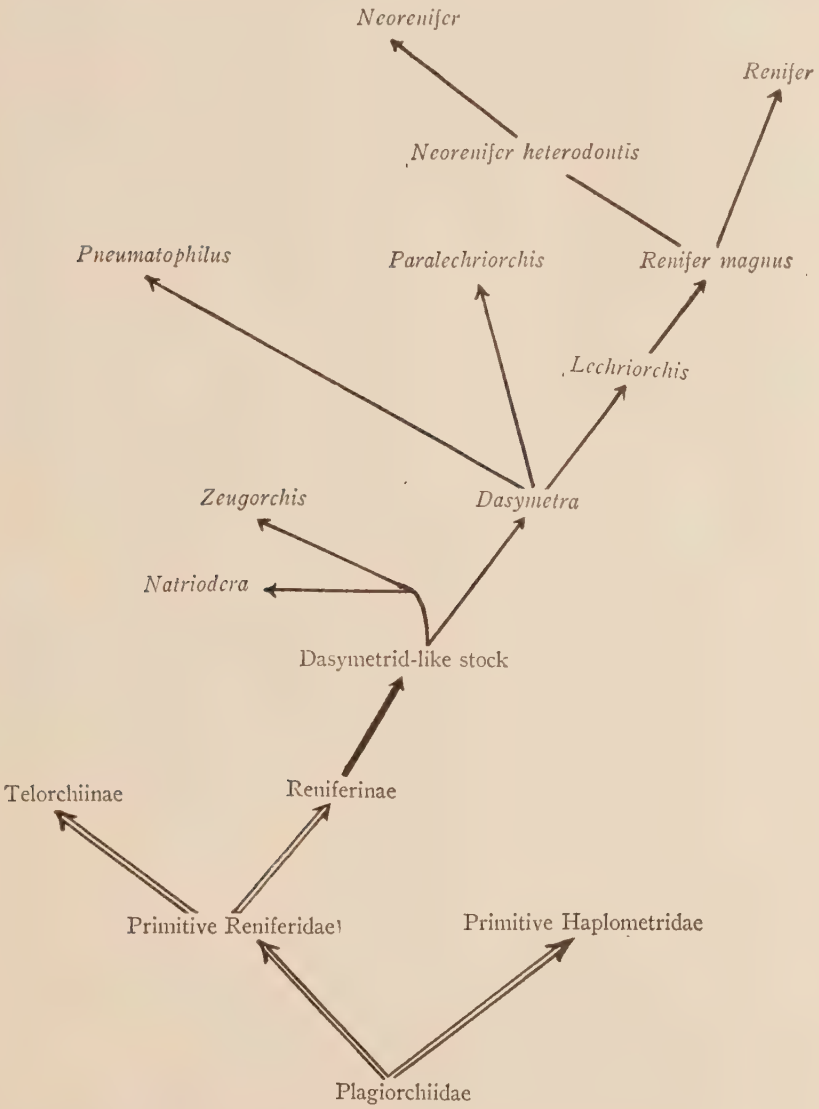
DISCUSSION

To the subfamily RENIFERINAE as diagnosed in the present paper belong eight genera and thirty-six species, two genera and seven species of which are described as new. In order to allocate properly the species and to clear up some of the confusion resulting from previous misinterpretation of the genus *Renifer* it has been necessary to considerably alter the diagnosis of certain genera belonging to the subfamily. This confusion has been augmented somewhat through a lack of knowledge of certain other genera, especially *Lechriorchis* and *Zeugorchis*. Price (1936) has cleared considerably the confusion regarding these two genera by redescribing the type species of each. With an understanding of these two genera and working on the assumption that the position of the genital pore, the degree of development of the metraterm and cirrus sac, the nature of the vitellaria, the position of the reproductive organs, etc., are characters of sufficient importance on which to base generic diagnoses,

we are able to more firmly establish the genera belonging to the subfamily.

From a phylogenetic standpoint we agree with McMullen (1937) in that the families RENIFERIDAE and PLAGIORCHIIDAE are closely related and that both should be included in the superfamily PLAGIORCHIOIDEA. Our study of the subfamily RENIFERINAE indicates that the family RENIFERIDAE has evolved from the family PLAGIORCHIIDAE through some primitive form closely allied to the genus *Plagiorchis*. Such a primitive plagiorchiid possessed typical characters in that the ovary was to one side of the midline just posterior to the acetabulum, the testes post-ovarian, the genital pore pre-acetabular, the vitellaria situated in the central body third, and the excretory bladder Y-shaped with the cornua reaching to or around the acetabulum. In all probability the main collecting tubules of the excretory system terminated the cornua. From such a primitive form we are able to derive the stock giving rise to the families RENIFERIDAE and HAPLOMETRIDAE of the superfamily PLAGIORCHIOIDEA through a lengthening of the cornua of the excretory bladder to the point that these completely encircle the acetabulum. From this point we find the main collecting tubules shifting from their terminal positions to a lateral position on the cornua in the reniferid-like stock. It would seem, therefore, that the RENIFERIDAE and the HAPLOMETRIDAE families arose from a common ancestor, as an offshoot from the primitive plagiorchid stock. (Text fig. 1.)

From the primitive reniferid fluke, which in our opinion somewhat closely simulated the body plan of the genus *Dasymetra,* we are able to derive the two subfamilies, RENIFERINAE and TELORCHIINAE, of the family RENIFERIDAE, the two subfamilies forming as independent offshoots from a common stock. From the primitive dasymetrid-like stock we derive the genus *Dasymetra* (Fig. 3) in which the vitellaria is situated ventral, lateral, and dorsal to the ceca in the central third of the body and the cornua of the excretory bladder fail to encircle the acetabulum completely. These two features together with the general topography of the body would seem to relate more closely the genus *Dasymetra* with the PLAGIORCHIIDAE than is observed for the other genera of the subfamily group. From this same stock, arising as an offshoot from the dasymetrid-like stock, we derive the genus *Zeugorchis* (Fig. 2). This has been through a shifting of the testes from their position just posterior to the ovary to a more posterior position, *i.e.,* to near the end of the body. The shifting of the testes initiated in the zeugorchid stock has given rise to a divergent series in which the ovary and vitellaria as well as the testes have become involved. Thus from the zeugorchid branch we derive the genus *Natriodera* (Fig. 1) in which the ovary, testes, and vitellaria have come to occupy positions midway between the end of the body and ace-



TEXT FIG. 1. Schematic diagram of possible phylogenetic relationship of genera of the subfamily RENIFERINAE, with an indication of the relationship of the family RENIFERIDAE to the PLAGIORCHIÍDAE and HAPLOMETRIDAE.

tabulum. The characters of the genus *Natriodera* show evidence of forming a parallel series in the subfamily RENIFERINAE that simulates the body plan of the subfamily TELORCHIINAE.

With slight modifications in the body plan of the *Dasymetra* group we derive the genus *Lechriorchis* (Fig. 4). The genus *Pneumatophilus*

(Fig. 5) is originated from the lechriorchid stock as an independent offshoot, being evolved through a series of forms that broadened the posterior half of the body to accommodate the elongation and subsequent coiling of the uterus. A second offshoot from the lechriorchid stock showing a reduction in the length of the cirrus sac and a tendency to overdevelop the metraterm, especially its length, has given rise to the genus *Paralechriorchis* (Fig. 6). Through a shifting of the position of the genital pore from its median position in the lechriorchid stock to a lateral position we derive the reniferid series. This shift in the position of the genital pore has been accomplished in some such form as *Renifer magnus* (Figs. 7 and 11) in which we find typical lechriorchid characters except for the position of the genital pore, which has moved from its position on the midline behind the bifurcation of the ceca to a position outside the area between the fork of the ceca and acetabulum and has come to occupy a lateral position on level with the fork of the ceca. It is through some such form as *Renifer magnus* that we derive the genus *Renifer* (Fig. 8) from the lechriorchid stock. Branching from the *Renifer magnus* type we trace the further migration of the genital pore to the region of the pharynx and oral sucker. *Neorenifer heterodontis* (Figs. 9 and 17) and *N. elongatus* furnish us with evidence of this migration of the genital pore, for it is in forms such as these that we see the genital pore migrating from the median position to the lateral position, through the *Renifer magnus* type, then passing gradually from its position near the level of the bifurcation of the ceca to the level of the pharynx or oral sucker. Thus we derive the genus *Neorenifer* (Fig. 10) from the reniferid stock through *Neorenifer heterodontis* and *N. elongatus*.

SUMMARY

1. A review of the systematics of the subfamily RENIFERINAE of the family RENIFERIDAE is presented.

2. A diagnosis of the subfamily RENIFERINAE is proposed in which both larval and adult characters are used as a basis of classification.

3. Diagnoses based on concrete anatomical characters are proposed for the genera included in the subfamily RENIFERINAE.

4. Two new genera, *Neorenifer* n. g. and *Paralechriorchis* n. g., and seven new species, *Renifer magnus* n. sp., *R. laterotrema* n. sp., *Lechriorchis abduscens* n. sp., *Neorenifer georgianus* n. sp., *N. glandularis* n. sp., *N. drymarchon* n. sp., and *N. heterodontis* n. sp., are proposed as new to the subfamily.

5. The allocation of species to the genera *Renifer* and *Neorenifer* is at variance with previous classifications of these genera.

6. A key to the genera and species of the subfamily is presented.

7. A possible phylogenetic relationship of the genera of the subfamily RENIFERINAE is discussed.

8. A schematic diagram of the phylogenetic tree of the subfamily RENIFERINAE is appended.

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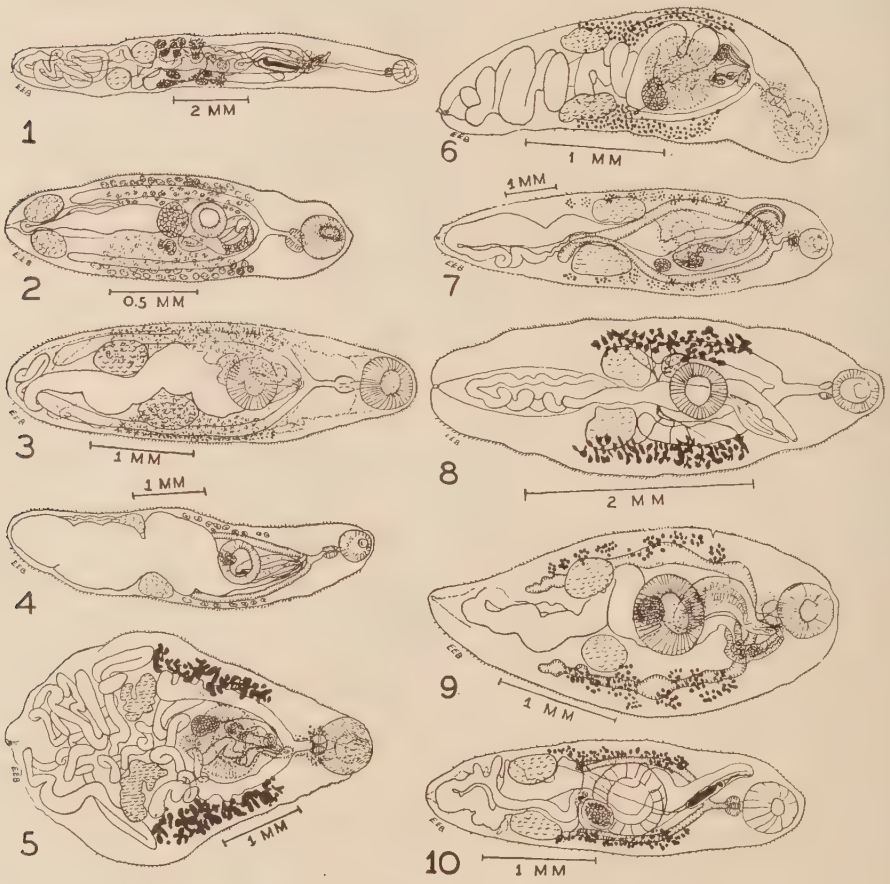

PLATE I

(Original figures drawn with the aid of the camera lucida)

FIG. 1. *Natriodera verlatum* (Talbot). Photographed and reduced for reproduction. Dorsal view. (After Talbot).

FIG. 2. *Zeugorchis aequatus* Stafford. Ventral view. (After Price).

FIG. 3. *Dasymetra conferta* Nicoll. Photographed and reduced for reproduction. Ventral view. (After Nicoll).

FIG. 4. *Lechriorchis primus* Stafford. Ventral view. (After Price).

FIG. 5. *Pneumatophilus leidyi* Byrd & Denton. Photographed and reduced for reproduction. Dorsal view. (After Byrd & Denton).

FIG. 6. *Paralechriorchis syntomentera* (Sumwalt). Dorsal view. (Original).

FIG. 7. *Renifer magnus* n. sp. Dorsal view. (Original).

FIG. 8. *Renifer ellipticus* Pratt. Ventral view. (After Pratt).

FIG. 9. *Neorenifer heterodontis* n. sp. Ventral view. (Original).

FIG. 10. *Neorenifer orula* (Talbot). Dorsal view. (After Talbot).

PLATE II

(All figures drawn with the aid of the camera lucida)

FIG. 11. *Renifer magnus* n. sp. Dorsal view. (Original).
FIG. 12. *Renifer laterotrema* n. sp. Ventral view. (Original).
FIG. 13. *Lechriorchis abduscens* n. sp. Ventral view. (Original).
FIG. 14. *Neorenifer georgianus* n. sp. Ventral view. (Original).
FIG. 15. *Neorenifer glandularis* n. sp. Dorsal view. (Original).
FIG. 16. *Neorenifer drymarchon* n. sp. Ventral view. (Original).
FIG. 17. *Neorenifer heterodontis* n. sp. Ventral view. (Original).

DESCRIPTION OF *RHABDOCHONA OVIFILAMENTA* N. SP. (NEMATODA: THELAZIIDAE) WITH A NOTE ON THE LIFE HISTORY*

THOMAS H. WELLER

On August 5, 1937, during the examination of perch obtained from the commercial fisheries in Cheboygan, Michigan, the intestine of one fish was found to contain four mature nematodes, apparently belonging to the genus *Rhabdochona* Railliet (1916) of the family THELAZIIDAE Railliet (1916). The fish were caught just north of Big Stone Bay in the Straits of Mackinaw in Michigan. In the examination of 136 perch from this locality and of 519 perch from the inland lakes of northern Michigan during the summers of 1936 and 1937, no other examples of this genus were collected. This nematode appears to be a new species and for it the name *Rhabdochona ovifilamenta* is proposed. Measurements given below are based on toto mounts of one male and three mature females, the material having been fixed in hot formalin-acetic-alcohol and stained with Semichon's acetic-carmine.

Rhabdochona ovifilamenta n. sp.
(Figs. 1–7)

Specific diagnosis: Rhabdochona. Small slender worms, tapering at both ends, with smooth and unstriated cuticula. Anterior end slightly rounded, 0.024–0.034 mm in diameter. Two rudimentary lateral lips. Prostom containing eight minute teeth which originate from longitudinal ridges on the thick chitinous wall. Prostom cyathiform; mesostom long and narrow; at base of prostom are four minute chitinous teeth, one dorsal, one ventral, and two lateral. Esophagus indistinctly divided into short anterior muscular portion and long posterior glandular portion, with prominent group of glands around base.

Male: 6.9 mm in length and 0.13 mm in width at midpoint; stoma, from anterior end to base, 0.11 mm long; nerve ring, 0.13 mm from anterior end; width of body at nerve ring, 0.05 mm; excretory pore, 0.23 mm from anterior end; base of anterior muscular portion of esophagus 0.40 mm from anterior end; base of posterior glandular portion of esophagus 2.10 mm from anterior end; anus, 6.57 mm from anterior end; width at anus, 0.09 mm; tail 0.33 mm long, conical, recurved, with small spine-like process at tip; spicules very unequal and dissimilar; large spicule, elongated and arcuate, with proximal end cephalated, measuring 0.40 mm long by 0.01 mm wide at midpoint, and bearing prominent groove which extends from point just posterior of midpoint to distal end where floor of channel is prolonged into sharp spine; small spicule, slightly curved, distal end blunt, measuring 0.044 mm long by 0.006 mm wide; gubernaculum, scoop-shaped, heavily chitinized, about 0.07 mm long; genital papillae, composed of nine pairs of (double?) pre-anal papillae and five pairs of single post-anal papillae; caudal alae very narrow; testes, extend a slight distance anterior to midpoint of the body and are but slightly convoluted.

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* Contribution from the University of Michigan Biological Station.

The writer wishes to express his appreciation to Dr. Lyell J. Thomas, under whose direction this work was carried out, for his many helpful suggestions and criticisms.

Female: 13.3 mm long; stoma, from anterior end to base, 0.13 mm; nerve ring, 0.19 mm from anterior end, placed slightly obliquely; excretory pore, 0.33 mm from anterior end; base of anterior muscular portion of esophagus, 0.44 mm from anterior end; base of posterior glandular portion of esophagus, 2.75 mm from anterior end; vulva, 7.10 mm from anterior end of body, has two slight prominences; width of body at vulva, 0.24 mm; vagina, thick, tubular, extending posteriorly about 0.33 mm; anus, 12.95 mm from anterior end; width at anus, 0.07 mm; tail straight, with small blunt spine at tip; eggs averaging $34 \times 19\ \mu$ with thick transparent shell, embryonated when laid; eggs when laid covered with many filaments, which while concentrated at the poles, originate over the whole surface.

Host: Perca flavescens Mitchill, the yellow perch.

Location: Intestine.

Type locality: Big Stone Bay, Straits of Mackinaw, Michigan.

Type specimen: U. S. Nat. Mus. Helm. Coll. No. 9074.

Due to the fact that only one male was found, there is some doubt as to the exact structure of the anal papillae. The pre-anal papillae (Fig. 7) appear paired, but this point requires confirmation when more material is available.

R. ovifilamenta is the first member of this genus for which filamentous eggs have been reported. The eggs, when laid, are covered with a varying number of fine filaments, most of which are concentrated at the poles, but which also originate over the whole surface (Fig. 8). The filaments are very similar to those of *Cystidicola farionis* as described and pictured by Van Den Berghe (1935). Such filaments are not visible *in utero,* and it is possible that they have been overlooked in some of the other members of this genus.

As far as can be determined from the literature available, twenty-two species of *Rhabdochona* Railliet (1916) have previously been described. *Rhabdochona ovifilamenta* is closely related to *R. opienensis* Hsü (1933) in that they both have a wide anterior prostomal chamber (Fig. 3) which has four small chitinous teeth at its base. Similar teeth have been described from no other members of this genus. *R. ovifilamenta,* however, differs from *R. opienensis* in having smaller spicules, a shorter esophagus, a different number of anal papillae, and in the more cephalad position of the nerve ring. *R. paski* Baylis (1928) also has a widened anterior chamber, but the fact that it lacks the basal teeth, has many more anal papillae, shorter spicules, and is twice as large distinguishes this form from *R. ovifilamenta.*

The large spicule of *R. anguillae* Spaul (1927) is much like that of *R. ovifilamenta* showing the same channeling in the posterior half. *R. anguillae* differs in being much larger, having larger eggs, and in having a different arrangement of the anal papillae.

R. cascadilla Wigdor (1918), the only species previously described from the United States, has a smaller number of anal papillae and is further distinguished from *R. ovifilamenta* by the fact that the large spicule is much shorter, being only one-tenth as long. *R. zacconis*

Yamaguti (1935), *R. amago* Yamaguti (1935), and *R. girellae* Yamaguti (1935) are all larger than *R. ovifilamenta,* and differ also in not having the cuticular stomal ribs that are characteristic of most of the members of this genus. *R. zacconis* and *R. amago* have twelve teeth in the prostom, as compared to the eight teeth found in *R. ovifilamenta,* while similar teeth are absent in *R. girellae*. *R. gymnocrani* Yamaguti (1935) and *R. salvelini* Fujita (1927) have much larger eggs than those of *R. ovifilamenta*. *R. salvelini* also differs in having symmetrical spicules. *R. gymnocranii* further differs in having only two prostomal teeth.

R. macrolaima Gendre (1921) and *R. gambiana* Gendre (1921) differ from *R. ovifilamenta* in having twelve prominent ribs in the prostomal cavity in contrast to the eight small ribs found in the latter. No male specimen of *R. macrolaima* has been found; the female differs further in having a tail that is proportionately much shorter. *R. gambiana* also differs in having a greater number of pre-anal papillae and in having peculiar lateral processes on the eggs. *R. acuminata* (Molin, 1860) Gendre (1921) may be distinguished from *R. ovifilamenta* by the presence of fourteen prominent ribs in the prostom and the greater number of anal papillae.

R. ovifilamenta differs from *R. denudata* (Dujardin, 1845) Railliet (1916) in size and in the number of anal papillae. *R. uca* Pearse (1932) described from one female, has a relatively longer esophagus and longer tail than has *R. ovifilamenta*. *R. kidderi* Pearse (1936) is different from *R. ovifilamenta* in that the large spicule is much shorter and in the fewer anal papillae. In the description of *R. kidderi,* the egg measurements are given as 0.14 by 0.022 mm; this is apparently a typographical error. The type material of this form was examined and the average measurement of twenty-five embryonated eggs *in utero* was found to be 0.014 by 0.030 mm.

R. elegans Travassos, Artigas and Pereira (1928) differs from *R. ovifilamenta* in being larger, having a greater number of anal papillae, and in the spicules. *R. conoura* (Linstow, 1885) Chitwood (1933) differs from *R. ovifilamenta* in having fewer preanal papillae, and by the fact that the large spicule is much shorter. *R. hellichi* (Sramek 1901) Chitwood (1933) differs in having similar spicules and in the number of preanal papillae. The presence of two prominent lateral papillae near the anterior end, and differences in the size of the eggs and spicules, distinguish *R. fortunatowi* Dinnik (1933). *R. turkestanica* (Skrjabin 1917) Yorke and Maplestone (1926) of which only the female is known, appears to differ in the relative length of the tail and the position of the excretory pore.

Descriptions of two forms, *R. savini* and *R. gnedini,* attributed to Skrjabin, have not been available for comparison. At the present time

it is not possible to devise a satisfactory key for the members of this genus, due to inadequacies existing in the descriptions of the forms.

Note on the Life History

The eggs of *R. ovifilamenta* are embryonated when laid. When kept in tap water at room temperature for several days there was no sign of further development or hatching. Using the limited supply of eggs one infection experiment was set up. On August 6, 1937, eggs were placed with one *Gammarus* sp., one *Hyalella knickerbockeri*, and two unidentified chironomid larvae. These were examined on the 12th of August and nine motile first stage larvae were found attached to the intestinal wall of *Hyalella*. The chironomids and *Gammarus* were negative. The first stage larvae (Fig. 1) measured from 0.14 to 0.18 mm in length and about 0.012 mm wide at the midpoint, and appeared ready to molt. A small stylet was present in the buccal cavity. The nerve ring was about 0.02 mm from the anterior end, and the anus about 0.02 mm from the posterior end. The genital primordium was located just anterior to the midpoint of the body. While requiring confirmation, this finding would seem to indicate that *Hyalella knickerbockeri* may serve as the intermediate host of *R. ovifilamenta.*

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Explanation of Plate, p. 408

All figures were drawn with the aid of a camera lucida.

Fig. 1. First stage larva of *Rhabdochona ovifilamenta* from intestine of *Hyalella knickerbockeri*, in Ringer's solution.

Fig. 2. Anterior end of female, toto mount.

Fig. 3. Detail of mouth region, toto mount.

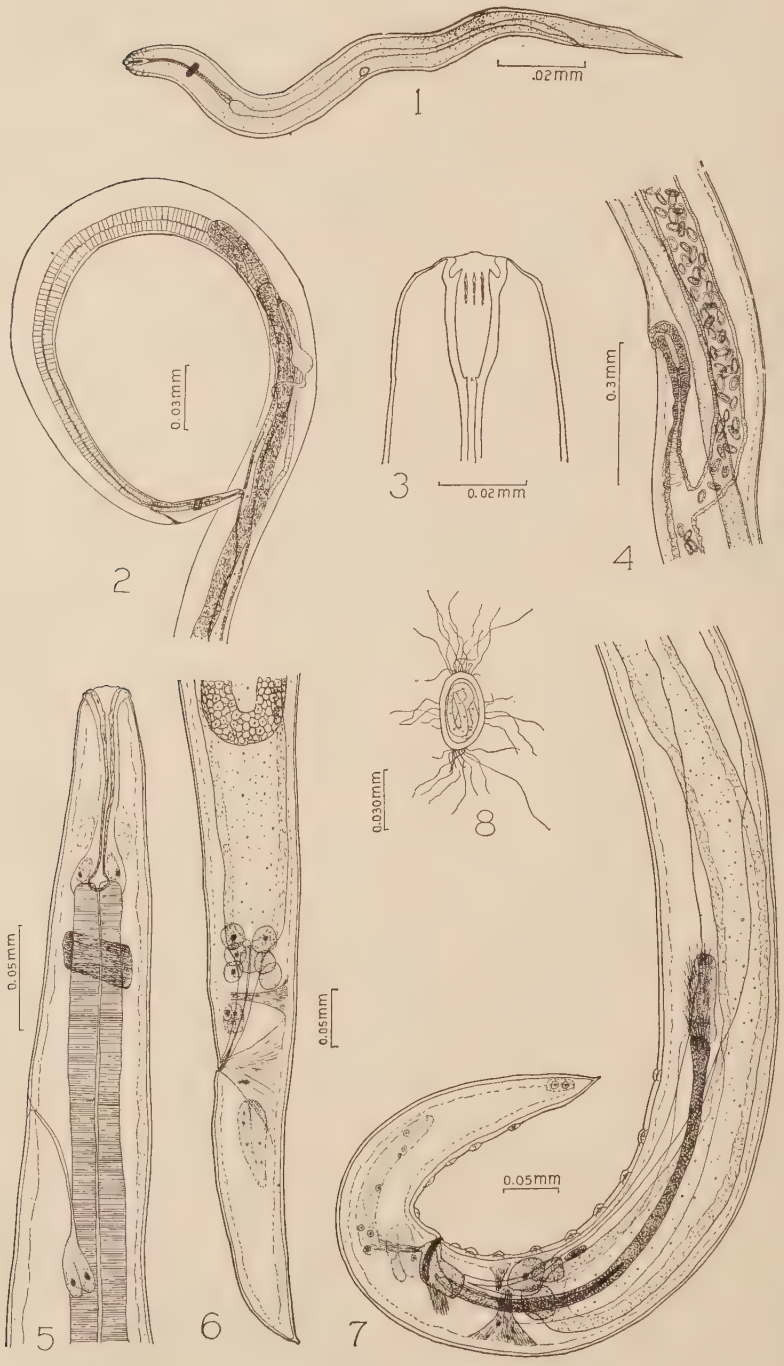
Fig. 4. Region around vulva, showing vagina, ovejector, and beginning of uteri. Drawn from living specimen.

Fig. 5. Anterior end of male, toto mount.

Fig. 6. Posterior end of female, toto mount.

Fig. 7. Posterior end of male, toto mount.

Fig. 8. Egg, shortly after being laid.



Rhabdochona ovifilamenta

OBSERVATIONS ON THE LIFE HISTORY OF *RAILLIETINA ECHINOBOTHRIDA* AND OF *R. TETRAGONA* (CESTODA)

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INTRODUCTION

The life histories of the two common poultry cestodes, *Raillietina echinobothrida* and *R. tetragona* have been puzzling to parasitologists for many years, because all attempts to demonstrate them and to repeat published results have been unsuccessful. The only verified information on these life histories was published by Jones and Horsfall (1935) who reported that two species of ants serve as intermediate hosts for these cestodes.

The present paper gives descriptions of the habits of the ants, inconclusive experimental infections of ants, seasonal distribution of the infected ants, distinguishing features of the larval and adult worms, geographical distribution of the intermediate and final hosts, as well as information having a direct bearing on the stages of the life cycle of the two tapeworms.

These two species of cestodes have been confused in literature because their diagnostic characters are inconspicuous and not easily distinguished, and because their distribution is similar. In this paper the cestodes will be treated as two distinct but closely related species, in spite of the fact that their life histories appear to follow such identical patterns that it was occasionally impossible to distinguish the larval forms by means of known characters.

These tapeworms are of considerable economic importance. They injure the host by destroying tissue of the intestinal wall, by burying their heads deep in the wall and even through the muscularis mucosae, and by causing the formation of intestinal nodules.

HISTORICAL

Piana (1881) described two cysticercoids from a snail, *Helix* sp., which he designated as larvae of *Taenia botrioplites,* a species since shown to be synonymous with *Raillietina echinobothrida.* Since the description of the cysticercoid given by Piana is inadequate for a specific determination, and since all attempts to infect experimentally or to find naturally infected mollusks have been unsuccessful, these cysticercoids are not considered to be larvae of *R. echinobothrida.* Ackert (1919)

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found *R. tetragona* in chickens which had been fed several thousand house flies, *Musca domestica.* The flies had been collected in yards containing chickens infected with *R. tetragona.* The experimentally fed birds were kept under controlled conditions and, upon post mortem examination, three of the sixteen chickens were found to be infected with this cestode. Ackert's examination of the adult cestodes indicated that they were *R. tetragona.* He was not able to demonstrate the cysticercoids in naturally infected flies nor could he experimentally infect flies with this cestode. Attempts by the present writer to infect fly larvae or adult flies with *R. echinobothrida* and *R. tetragona* were unsuccessful.

Joyeux (1927) tried direct feeding experiments with negative results. Lopez-Neyra (1930, 1931) reported feeding a five-day-old chick gravid segments which had been collected from a bird infected with *R. echinobothrida, R. cesticillus,* and *Choanotaenia infundibulum.* When examined post mortem 3½ months later, the experimental bird contained many young specimens of *R. cesticillus* and numerous intestinal nodules in which were found small specimens (5–8 mm long) of *R. echinobothrida;* a control bird was negative. The following year the experiment was repeated using two chickens, one experimental bird and one control; only segments of *R. echinobothrida* were fed to the experimental bird. Forty days after feeding, the control was negative and the experimental bird contained numerous young specimens of *R. echinobothrida.* Jones and Horsfall (unpublished data) attempted to infect chickens by feeding them gravid segments of *R. echinobothrida* and *R. tetragona;* the results were negative.

Jones and Horsfall (1935, 1936) showed that the ants *Tetramorium caespitum* and *Pheidole* sp. were intermediate hosts for both *R. echinobothrida* and *R. tetragona.* The cysticercoids of the two species, which had been dissected from naturally infected ants, were measured, distinguished, and fed to laboratory-reared chickens. The experimentally fed birds, as well as controls, were kept under laboratory conditions to prevent extraneous infection. Three weeks after feeding the cysticercoids, adult *R. echinobothrida* and *R. tetragona* were recovered post mortem from the experimentally fed birds; the controls were negative

Joyeux and Baer (1937) reported the finding of the larvae of *Raillietina echinobothrida* in the naturally infected ant, *Tetramorium semilaeve,* in the region of Marseilles, France. The total length and width, rostellum, rostellar hooks, suckers, sucker hooks, and internal structures are described and measured.

METHODS

The investigation reported in this paper was carried on continuously from November, 1934, to May, 1937, at the National Agricultural Research Center, Beltsville, Maryland. The yard in which all observations

were made was 30 by 40 feet, and located on a gentle well-drained slope having sandy, gravelly soil, with no cover except one small oak tree that had been trimmed to reduce its shade. Five small decaying tree stumps stood just flush with the surface of the ground, and some debris, boards, sticks and burlap sacks were placed on the ground as cover for insects. This provided an ideal environment for insects which frequent bare, dry, sandy soil. This yard contained mixed groups of chickens of different ages and breeds, the birds being kept there for various periods. The following helminths listed in the order of their abundance were found in the chickens kept in this pen during the $2\frac{1}{2}$ year observation period: *Heterakis gallinae, Hymenolepis carioca, Ascaridia lineata, Raillietina cesticillus, R. echinobothrida, R. tetragona, Choanotaenia infundibulum, Davainea proglottina,* and *Hymenolepis cantaniana.* No attempt was made to control these helminths, and infected birds were put into this pen in order to introduce more parasites. There were always some chickens infected with *R. echinobothrida* or *R. tetragona* present in this yard and the gravid segments could be found in freshly passed feces at any time of the year.

At monthly intervals, various laboratory-raised chickens of different ages were placed in this yard. From 2 weeks to 3 months later the introduced birds, in groups of two or three were examined post mortem for helminths. All recovered worms were carefully identified and all specimens of *R. echinobothrida* and *R. tetragona* were preserved.

Invertebrates, particularly insects, were collected approximately twice a week from this pen during the spring, summer and fall and examined for cysticercoids under a dissecting microscope, or were fed undissected to laboratory-raised chickens. After August 16, 1935, when the cysticercoids first had been found in the ant *Tetramorium caespitum,* ants were collected frequently from this pen until the coming of cold weather, by means of a small exhaustor described by Wheeler (1932). When more ants were collected than could be examined in the available time, they were fed undissected to chickens.

The cysticercoids, which were always found in the anterior part of the gaster, were dissected from the ants in a drop of tap water or physiologic saline. Egg albumen as described by Krull (1934) was used as a mounting medium for the study of living larvae.

In order to observe the habits of the ants and to have on hand for experimental use an abundance of these insects during the winter when they were not available outside, 44 colonies were kept in the laboratory for different periods. Since *Tetramorium caespitum* was larger than *Pheidole vinelandica* and the more easily handled, 40 of the colonies contained only this species. Only workers, larvae, and pupae were kept in the laboratory colonies, since no queens and only 5 males of *T. caespitum*

were found after persistent searching in the experimental yard. The ants were divided into groups of 100 to 300 workers, with some pupae and larvae, all having been collected from the same colony. Each group was deposited in a large petri dish (15 cm in diameter) with a little sand or dirt to supply nest material. The petri dishes were placed in a half of a larger petri dish, the bottom of which was covered with water to provide a moat which discouraged the ants from leaving their colonies. Segments of *R. echinobothrida* or *R. tetragona* recovered by screening from the feces of infected chickens were placed on small pieces of paper towelling and introduced daily into the petri dish colonies. A variety of other foods such as chicken mash, fruit juices, sugar, etc., were also offered the ants.

EXPERIMENTAL DATA

The first clue to the intermediate hosts of *R. echinobothrida* and *R. tetragona* was discovered while the writer had under observation in the experimental yard several fecal samples containing *R. echinobothrida* proglottids. An ant carried one of these segments from the feces to an entrance to a nest and disappeared with it. Ants were then examined from this yard and all were found to be negative until August 16, 1935, at which time 3 *T. caespitum* were dissected and found to contain 4 cysticercoids the scoleces of which resembled those of *R. echinobothrida*. Later cysticercoids were recovered from both *T. caespitum* and *P. vinelandica* collected in the experimental yard. Of 1503 *T. caespitum* dissected, 2.5 per cent contained cysticercoids, while of 368 *P. vinelandica* examined, 5.9 per cent harbored cysticercoids. The average number of larval tapeworms found in one ant was 2.1 and the maximum number was 9.

When the larvae, pupae and workers from the experimentally fed colonies were examined, one cysticercoid of *R. echinobothrida* was found in each of two workers of *T. caespitum*. It is possible that the ants might have been infected when collected 9 months before. This however does not seem probable as both appeared to be young ants. Since all attempts to repeat the experimental infections were unsuccessful, the writer does not consider them a conclusive experimental demonstration.

A total of 12 chickens were fed cysticercoids promptly after they had been dissected from naturally infected ants. The resulting infections are given in table 1.

Varying numbers of undissected ants collected in the experimental yard were fed to 23 chickens during 1935 and 1936. Nineteen of these birds became infected as shown either by the passage of gravid segments or by the presence of the tapeworm upon post mortem examination. It was usually necessary to feed 300 to 500 ants to a bird in order to obtain 10 to 15 cestodes, but on one occasion 1 specimen was recovered from a chicken fed only 14 specimens of *P. vinelandica*.

TABLE 1.—*Number of adult cestodes recovered from chickens fed cysticercoids of* R. echinobothrida *or* R. tetragona

Chicken	Cysticercoids fed	Cestodes recovered
237	12	8
090	9	6
275	6	1
297	4	1
258	3	2
266	3	2
231	2	1
208	2	1
259	2	1
857	1	1
250	1	0
279	1	0
Total	46	24

Before the discovery of the cysticercoids in the ants, it had been noted that chickens in the experimental yard became infected with *R. echinobothrida* and *R. tetragona* only during certain seasons. In order to determine the limits of these periods, chickens were placed in the experimental yard at intervals throughout the year. During 1935, a total of 42 chickens were placed in the yard; the first to be found infected had been in the inclosure from May 20 to July 10, 1935, and the last from December 8, 1935, to January 7, 1936, the dates May 20, 1935, to January 7, 1936, marking the extreme limits of the infection period for that year. Since the chickens were in the experimental yard for a month or more during which time they could have eaten infected ants, it is likely that the infection period was approximately June 1 to December 31, 1935. During the aforementioned period, 31 uninfected birds were placed in this pen, 22 of which contained *R. echinobothrida* and *R. tetragona* at autopsy. In 1936, 76 chickens were left in the experimental pen for various intervals; the first bird to be found infected had been there from June 2 to June 28, 1936, the last from October 5 to November 13, 1936. Forty-eight of the birds were examined during this period of possible infection, and of these 25 were infected. Between January 1 and May 15, 1937, 19 birds were placed in the pen and none of them contained either species of tapeworm as determined by post mortem examination. These figures indicate definitely that chickens in the experimental yard became infected only during the latter half of the year, June 1 to December 31.

Under the existing conditions it was not practical to determine accurately the longevity of these parasites in chickens in the experimental yard, but it is certain that some of them lived through the winter because of the presence of the segments in the feces at all times of the year. Naturally and experimentally infected chickens kept in batteries in the laboratory usually lost their infection in 4 months. In two birds gravid segments were still present in the feces 11 months (chicken 239) and 8 months (chicken 300), respectively, after infection. Chicken 239 passed

a small number of segments of *R. echinobothrida* (30 to 40 daily while under observation in the laboratory), indicating only a light infection. This hen was put in the experimental pen July 6, 1936, after having passed proglottids for 11 months. When examined post mortem 2 months later, September 16, 1936, the bird contained 300 *R. echinobothrida* and *R. tetragona,* showing that she had been heavily reinfected.

OBSERVATIONS ON ANTS

Three species of ants were found in the experimental chicken yard as follows: a small black species, *Pheidole vinelandica;* the pavement ant, *Tetramorium caespitum,* which is a medium-sized dark brown form; and *Prenolepsis* sp., a large golden brown species. The last mentioned could not be experimentally infected nor was it found naturally infected with tapeworm larvae, and is not included in the following observations.

For their first seasonal appearances ants were observed moving about in the experimental yard on March 15, 1935, and on March 16, 1936. No records were kept of the last seasonal appearance of either ant, although they were seen in December. Ants were active at times in this yard during every month of the year except January and February, yet chickens became infected with *Raillietina echinobothrida* and *R. tetragona* only during the latter half of the year.

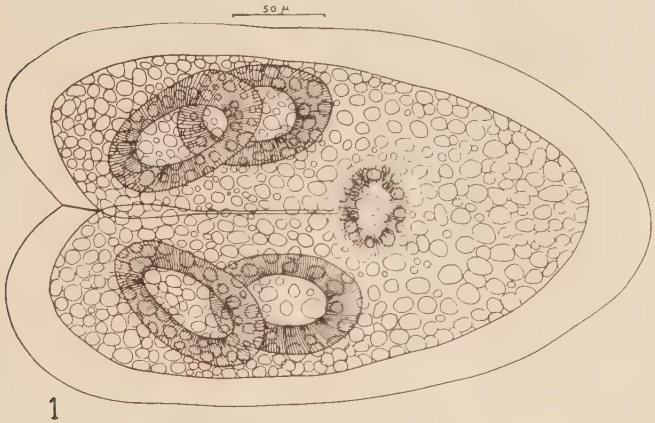
A study of the habits of these ants under natural conditions did not throw much light on how infection with the cysticercoids takes place. According to Donisthorpe (1937), *T. caespitum* stores grain and seed; the young larvae are fed on disgorged food and the older larvae on solid substances. Smith (1918) reports that *P. vinelandica* stores seeds but does not mention the kind of food given the larvae. The writer was unable to observe the feeding of the immature stages in the laboratory colonies because all larvae were hidden in the galleries or were abandoned if exposed. The worker ants readily took the gravid segments placed in their nests, carried them around and eventually transported them into the covered galleries.

Two species of guests were present in the laboratory colonies, red mites of the subfamily *Uropodinae* and springtails of the genus *Entomobrya,* but no larval cestodes were found in them.

CHARACTERISTICS OF THE LARVAL AND ADULT CESTODES

The cysticercoids and adults of *R. echinobothrida* and *R. tetragona* are similar morphologically and as each stage utilizes the same hosts, they are difficult to distinguish. The cysticercoids of the two species are so simple in structure that there are even fewer characters available for separating them than are available for separating the adults.

The cysticercoids are approximately elliptical in shape with the larval



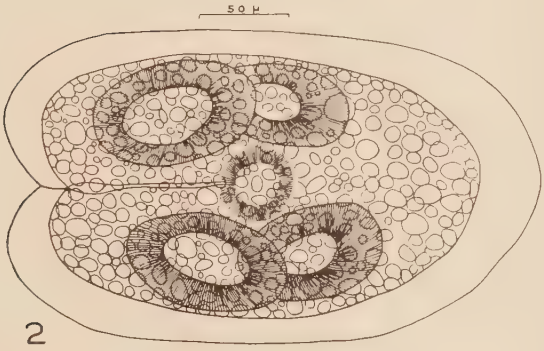


FIG. 1.—Diagrammatic drawing of a living cysticercoid of *Raillietina echinobothrida*, to scale.

FIG. 2.—Diagrammatic drawing of a living cysticercoid of *Raillietina tetragona*, to scale.

scolex invaginated into the bladder and entirely surrounded by a heavy cyst wall. Neither caudal appendage nor embryonal hooks were observed on any of the specimens examined. The larvae were filled with calcareous corpuscles which masked the internal structures. They were typical cysticercoids in all respects, without conspicuous distinguishing features. The measurements given in table 2 are those of living cysticercoids, the identifications of specimens being experimentally proved by feeding them to chickens and recovering the adults. The average sizes of these two species of cysticercoids are different but when the maximum and minimum measurements of each species are compared, there is some overlapping. In general the cysticercoids of *R. echinobothrida* are larger than those of *R. tetragona*. The measurements given for the length of the evaginated scolex, size of the suckers and diameter of the flattened

TABLE 2.—*Measurements in microns of living cysticercoids of* Raillietina echinobothrida *and* R. tetragona

Species	Entire cysticercoid			Scolex			Rostellar hooks		
	Maximum	Minimum	Average	Length	Suckers	Rostellar circle	Number	Rows	Length
R. echinobothrida	370 × 225	306 × 161	346 × 217	310–314	85–103 × 44–55	29–33	200	2	10–13
R. tetragona	306 × 166	289 × 161	293 × 165	243	103 × 59	25	100	1	6–8

rostellar circle were obtained from two larvae of *R. echinobothrida* and one of *R. tetragona,* consequently they can only be indicative of relative sizes. It is evident from these figures that none of the first six characters given in the table is sufficient for distinguishing these larval forms. The size, number of rows, and number of rostellar hooks are the only specific characters available. The rows of rostellar hooks lie so closely together that frequently they appear to coincide. In order to see the rows clearly, it was necessary to flatten the cysticercoids slowly in egg albumen. The difficulty in measuring and counting the hooks and the lack of other diagnostic characteristics make it extremely difficult to distinguish the two species.

The adults of *R. echinobothrida* and *R. tetragona* are so similar that they have undoubtedly been confused in some previous reports. The relative sizes of conspicuous structures are shown in table 3.

The character most satisfactory for determination of both cysticercoids and adults of the two species is the rostellar hook. Adult worms have as additional diagnostic characteristics the length of the cirrus pouch, location of genital opening, and shape of the extruded gravid segments in water. These characters may be variable depending upon contraction and expansion.

GEOGRAPHICAL DISTRIBUTION OF *R. echinobothrida* AND *R. tetragona* AND THEIR ANT HOSTS IN THE UNITED STATES

While both *R. echinobothrida* and *R. tetragona* are reported as widespread in the United States there are few statements to indicate which regions contain infected birds. Records of the occurrence of these cestodes have been published from the following states: New York, Maryland, District of Columbia, Illinois, Kansas, Nebraska, and Texas.

The distribution in the United States of *T. caespitum* and *P. vinelandica,* the ants serving as intermediate hosts of the tapeworms mentioned above, is indicated on the map, figure 3. This information was secured from state entomologists in scattered states, from the U. S. Bureau of Entomology and Plant Quarantine and from Prof. M. R.

TABLE 3.—*Diagnostic and comparative characteristics of adult* Raillietina echinobothrida *and* R. tetragona*

Species	Strobila		Scolex								Segments		
				Rostellum				Suckers					
	Length mm.	Width mm.	Diameter μ	Diameter μ	Number of hooks	Rows of hooks	Size of hooks μ	Diameter μ	Rows of hooks	Size of hooks μ	Cirrus pouch length μ	Genital aperture	Shape of gravid segments in water
R. echinobothrida .	250	1–4	250–450	100–150	200	2	10–13	90–200	8–12	5–15	130–200	Lateral anterior half of segments	Square; corners rounded
R. tetragona	250	1–4	175–350	30–70	100	1	6–8	50–150	8–12	3–8.5	60–100	Lateral posterior half of segments	Dumbbell shaped

* Adapted in part from Lang (1929).

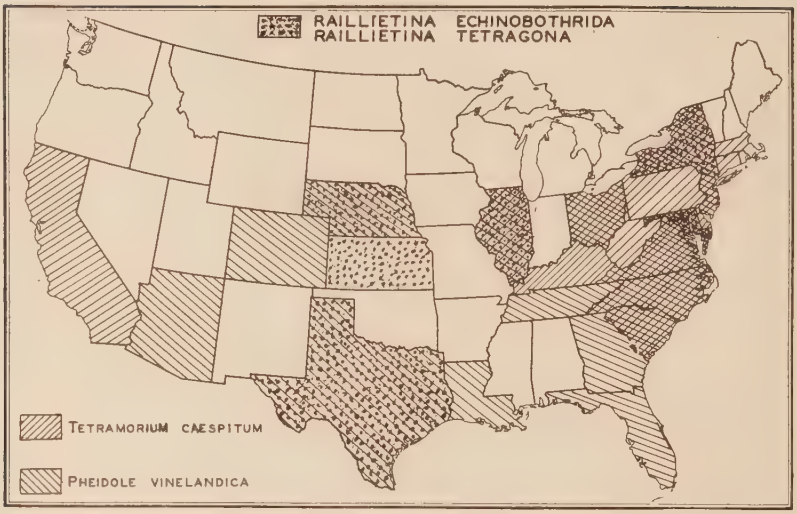


FIG. 3.—Map showing known distribution of ants *T. caespitum* and *P. vinelandica* and of cestodes *R. echinobothrida* and *R. tetragona* in the United States.

Smith of Mississippi. No records of either species were available from Minnesota, Montana, Oregon, or Washington, and records of *P. vinelandica* are also missing from Florida. Other blank states on the map indicate that either no authorities were consulted or that information from other sources was unavailable.

During August, 1936, 39 ants were collected in a chicken yard in Crete, Nebraska, by M. F. Jones, and forwarded to the writer. According to the late Prof. W. M. Wheeler of Harvard University, all of the specimens were *P. vinelandica.* Of these ants, two were infected with larvae of either *R. echinobothrida* or *R. tetragona,* one containing four cysticercoids and the other one. Owing to an error in technique during mounting, it was impossible to determine which of the two species had been collected.

DISCUSSION

The preceding data have definitely established under both natural and experimental conditions that chickens become infected with *R. echinobothrida* and *R. tetragona* as a result of eating ants which contain the cysticercoids of these two cestodes. The question remains of how the ants become infected with the larval cestodes. Fortunately seasonal data and observations on the life history and habits of the ants involved, give some clues to the means of infection. On different occasions ants in the experimental yard were observed carrying gravid segments of *R. echinobothrida* and *R. tetragona* into their nests. Since all attempts to follow the subsequent fate of these segments were unsuccessful, the

writer's idea of the possible means whereby the ants might be infected are discussed.

1. Donisthorpe (1927) and Smith (1918) reported that *T. caespitum* and *P. vinelandica,* respectively, store food in their subterranean galleries. If gravid segments of these two cestodes are stored, it is possible that the eggs might develop to a stage where they would be eaten by the ants. There have been no reports that such conditions of storage are necessary among any members of the family to which these cestodes belong. The writer has stored gravid segments of these two cestodes in the laboratory, refrigerator and in natural conditions, and with different combinations of temperature, humidity and light without ever finding any conditions in which the eggs remained alive for more than two days. This information does not eliminate the possibility that the storage of cestode eggs in the ant nest is necessary for the development of the cysticercoids, but it does cast considerable doubt upon it.

2. *T. caespitum* and *P. vinelandica* harbor guests and parasites in their colonies which might serve as secondary intermediate hosts for these cestodes. These extra-colony members could eat the segments carried into the galleries by the workers, thereby becoming infected. If this method is used the worker ants must become infected from the extra-colony members while the cysticercoids are still able to migrate through body tissue, because the larval cestodes were never found in the digestive tract but always free in the body cavity of the ant, a location to which they would need to move. In all the colonies examined from the experimental pen, only a few specimens of two species of guests were found, and all of these were negative for cysticercoids. There is no evidence either in the literature or from observations that these ants eat other inhabitants of their nests. This method of infection necessitates a rather complicated life cycle, for which there is no evidence at present.

3. Since practically all ants observed to be infected were worker ants (except two soldiers of *P. vinelandica*), it would be logical to expect that this caste ate the eggs, thus becoming infected. Developing cysticercoids in general may be distinguished from infective cysticercoids by the lack of characteristic rostellar hooks and of internal differentiation. More than 1800 workers were dissected, none of which contained developing cysticercoids. If such non-infective forms were present in the workers it would be logical to expect that some of them would have been found during the $2\frac{1}{2}$ year observation period, but none were.

4. The immature ants are the remaining group of colony inhabitants which might become infected in the nest. Of the three developing stages, eggs, larvae and pupae, only the larvae eat or are fed so they are the only stage which could eat the cestode eggs. The eggs of the gravid seg-

ments of the cestodes might be fed to or eaten by the larvae and they would develop during the larval and pupal stages, so by the time the workers emerged and were active on the surface of the ground, the cysticercoids would be infective. A few larvae and pupae from the infected nests were dissected, none of which contained any cysticercoids, but the number of them was too small to draw any conclusions.

Circumstantial evidence in favor of this last method of infection is apparent in the seasonal data. In the laboratory, chickens retained *R. echinobothrida* and *R. tetragona* for nearly a year and in the experimental pen their gravid segments were found at all seasons of the year, indicating that the eggs may be available to the intermediate hosts at all times. *T. caespitum* and *P. vinelandica* were only active and feeding at the surface of the ground in the experimental pen from about March 15 to December 31. It was during this period then that the workers must have collected the freshly passed segments. Examination of ants and chickens from the experimental pen showed that the ants containing infective cysticercoids were present approximately from June 1 to December 31.

This information on seasonal distribution shows that the ants found infected the first part of June must have acquired the infection since March 15 of the same year, or carried it over from the preceding year. If the latter circumstance were true, some infected ants should have been found in the collections made from March 15 to June 1, but instead they appeared only after the first of June. Donisthorpe (1927) reported that $2\frac{1}{2}$ to $3\frac{1}{2}$ months are necessary for the complete development of *T. caespitum* from the time the eggs are laid until the workers are active; no similar information was available for *P. vinelandica*. A $2\frac{1}{2}$ month minimum period for ant development corresponds to March 15 to June 1 during which the first new ants of the season might have developed. The similar intervals might easily be the time during which the larvae were infected with the cysticercoids and simultaneously the ants and cysticercoids developed. This $2\frac{1}{2}$ months between the first spring activity of the ants and the first finding of cysticercoids is well explained as the time during which the cysticercoids were developing in the larval and pupal stages in the subterranean galleries. This explanation also accounts for the lack of non-infective cysticercoids in worker ants.

Of the 4 possible methods of infection discussed, the last one seems the most probable explanation to the writer. There is no experimental proof for any of them, but there is circumstantial evidence in the form of seasonal data which supports the idea that the ants are infected in the larval stage and the cysticercoids and ants develop simultaneously.

SUMMARY

1. Two species of ants, *Tetramorium caespitum* and *Pheidole vinelandica,* have been demonstrated as intermediate hosts for *Raillietina echinobothrida* and *R. tetragona* in the United States.

2. Two specimens of *T. caespitum* were apparently infected with *R. echinobothrida* in the laboratory.

3. Under natural outdoor conditions, the cestodes were present throughout the year in chickens. The ants were active from March 15 through December, but the ants were only found naturally infected from approximately June 1 through December in both 1935 and 1936.

4. Both cysticercoids and adults of *R. echinobothrida* and *R. tetragona* can be distinguished only by the number of rows of hooks, the number of hooks and the size of the hooks in the rostellar crown.

5. The published records indicate that the two species of cestodes are found generally along the Atlantic Coast, Gulf of Mexico and in the Mississippi Valley. The species of ants serving as intermediate hosts are more widely distributed than the cestodes, having been found in more states of the previously mentioned regions and in a few western states.

6. While the observations on the life histories of these cestodes do not include the developing stages of the cysticercoids in the ants, they do tend to limit the time and place of this development to the ant nest.

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OBSERVATIONS ON THE BIOLOGY OF THE POULTRY CESTODE *DAVAINEA PROGLOTTINA* IN THE INTESTINE OF THE HOST

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In the course of some experiments dealing with the anthelmintic treatment of tapeworm-infected birds, it became necessary to estimate the degree of infection in the living birds. The data presented in this paper were obtained in the course of those studies.

White Leghorn chickens were experimentally infected with *Davainea proglottina* by feeding them garden slugs (*Agriolimax agrestis*) containing mature cysticercoids. The slugs had been fed gravid segments of the tapeworm three to four weeks previously. Infection in the birds was proven by the finding of gravid segments in their feces. The feces were collected by placing the birds in separate screen-bottomed cages which rested over pans containing enough 1½ per cent formalin solution to cover all the droppings passed in 24 hours. At the end of that time all the material was collected and allowed to settle in tall jars. The supernatant fluid was discarded and the sediment examined in small portions with a wide-field microscope until all of the segments had been counted.

A simple technique was used for counting the tapeworms in the intestine. The bird is first starved for 18 hours. Then 60 cc of 5 per cent formalin solution at 45° C. is injected directly into the gizzard by means of a long, fairly pliable, hard rubber cannula and a rubber bulb. If the solution is injected slowly, all of it will go into the intestine. After about ten minutes the bird is destroyed and the intestine removed. Care is taken not to lose any of the contained fluid since many of the tapeworms will free themselves from the mucosa during this treatment. Two-inch lengths of intestine are slit open in a petri dish into which is poured enough water to cover the mucosa. The majority of the worms are fixed *in situ* and may be distinguished quite readily since the opaque, white segments stand out against the reddened villi. The worms, including the scoleces, are picked out of the mucosa quite easily with a pair of needles under a wide-field binocular microscope. After removing all of the worms that can be seen, a further check on very small ones is made by scraping the mucosa and examining the scrapings in a small amount of water.

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THE PERIODICITY OF SEGMENT DISCHARGE

Wetzel (1932) found that there was a daily rhythm in the segment discharge of birds parasitized with *D. proglottina*. He found that practically all of the proglottids were discharged between 12:00 noon and 5:00 PM, with a peak reached at 3:00 PM.

An experiment was set up to determine whether or not this periodicity was manifested by our birds. Seven birds were placed in individual cages and their feces were collected several times each day. Segment counts for each bird were done over a period ranging from five days to two weeks.

The average percentage distribution of the number of segments discharged by all the birds in this study, during the time intervals considered, were calculated with the following results:

Segments discharged between			9:00 AM	and	11:00 AM—	1.6%
"	"	"	11:00 AM	"	1:00 PM—	4.9%
"	"	"	1:00 PM	"	3:00 PM—	10.5%
"	"	"	3:00 PM	"	5:00 PM—	34.0%
"	"	"	5:00 PM	"	9:00 AM—	49.0%

A few isolated observations made during the evening indicated that the discharge of segments ceased between 7:00 PM and 9:00 PM and was not resumed until the following morning.

Our studies were made during the months of June, July, and August at which time the period of daylight ended at about 8:00 PM. Accordingly, it appears that approximately 80 per cent of the segments were discharged during the last five hours of daylight, and about 50 per cent during the last three hours. Wetzel's studies, on the other hand, indicated that the peak of segment discharge was reached about 3:00 PM and that segment discharge practically ceased at 5:00 PM. His studies were conducted during the short days of January, however, in which darkness falls at about five o'clock. It seemed, therefore, that the discrepancy between his work and ours could be reconciled, if it could be shown that the periodicity of segment discharge depended upon the feeding activities of the birds which, in turn, is influenced entirely by the period of daylight.

To clear up this matter, observations were made for three days on two parasitized birds during the month of December. The results (Table 1) are in full agreement with Wetzel's and show that the feeding habits of chickens, as affected by the length of day, has a marked effect on the time of the daily cyclic discharge of segments. Furthermore, it is evident that the amount of feces eliminated has no causal relationship to the number of segments passed.

Another experiment was set up to determine whether the time of cyclic segment discharge could be altered by changing the time of feeding

TABLE 1.—*The peak of segment discharge in December from chickens parasitized with* D. proglottina

Bird number	Days	Percentage of total daily segment discharge, 12 : 00 noon to 4 : 00 PM	Percentage of daily total weight of feces discharged during same period
998	1	93	34
	2	94	39
	3	90	32
971	1	93	36
	2	98	38
	3	87	35

of the birds. The day after the results outlined in Table 1 had been procured, birds 998 and 971 were placed in a completely darkened room from 8:00 AM to 5:00 PM, at which time electric lights were turned on and allowed to burn until 8:00 AM the next morning. This reversal of "daylight" hours was kept up for five days to accustom the birds to the changed conditions. The birds went to roost promptly at 8:00 AM and did not start feeding until 5:00 PM.

The effect on the time of segment discharge was marked. Birds 998 and 971 passed 4 and 0 per cent, respectively, of the total number of segments discharged in twenty-four hours between the hours of 10:00 AM and 6:00 PM, while 96 and 100 per cent, respectively, were discharged between 6:00 PM and 10:00 AM the following morning. Observations made every two hours during one 24-hour period showed that the birds passed 66 and 84 per cent, respectively, of the segments between 2:00 AM and 6:00 AM and that 25 and 4 per cent, respectively, were discharged between 6:00 AM and 8:00 AM. Segment elimination ceased after 10:00 AM. This experiment showed that the time of the cyclic daily discharge of segments is not due to any innate characteristic of *D. proglottina,* but depends entirely upon the time of feeding and activity by the host.

THE RELATIONSHIP BETWEEN THE DAILY SEGMENT DISCHARGE AND THE NUMBER OF TAPEWORMS IN THE INTESTINE

The total daily segment counts of a number of birds were determined. These birds were sacrificed later and the scoleces in the intestine were counted. Although the total number of segments passed by a bird in 24 hours varied somewhat from day to day, these variations decreased after the bird had become adjusted to the surroundings, feed, and handling. Data which illustrate this point will be found in Tables 2, 3, and 4. It will be noted in Table 2 that quite often the highest segment count (indicated by italics) was reached a considerable time after the initial counts were made. It may be that some birds take longer to adapt themselves to the experimental conditions than others. Additional factors governing segment production by *D. proglottina* will be discussed elsewhere in this report.

TABLE 2.—*Relationship between daily segment counts and the number of* D. proglottina *in the intestine. Highest daily count is given in italics*

Bird No.	Number of days on experiment														Number of scoleces found post morten
	1	2	3	4	5	6	7	8	9	21	31	68	96	115	
974	556	*633*	449	487	560										991
330	99	108	154	149	*181*										237
327	30	30	*31*	14	24										113
984	1207	*1259*	1118			1077									Count not made
972	69	*151*	134		132	144									304
889	409	490		521	632	*680*									665
887	59	*70*		57	69	56									164
388	18	23	18	31	30	*32*									31
331	13	14	11	7	7	*25*	15								28
340	44	20	13	47	45	48	46	*59*							100
986	14			40	41	41			33	*52*					43
809	36	156			123	46	66				*210*				676
807	7	43	43	41	89			125	133		*205*				264
977	636	610		670		667		567				*813*			1010
973	418	469			596	615	545						*667*		680
967	471	602		563		656		458						*767*	749
968	139		140	103	146	105								*383*	499

With only a few exceptions,[1] it was found that the largest number of segments passed in any one day never exceeded the number of scoleces found in the intestine. Most often the number of scoleces found was greater than the highest 24 hour segment count. In six instances (birds 889, 986, 967, 973, 388, and 331), the number of scoleces found in the intestine practically coincided with the largest number of segments passed in one day. This indicates that under optimum conditions nearly every tapeworm in the intestine produces one segment during each 24 hour period. It was also noted that in many instances, notably in those where the scolex count exceeded the highest segment count, scoleces were found in the intestine with only one, two, or three immature segments attached while, in the same intestine, numerous complete strobilae (seven segments) with gravid terminal proglottids were encountered. Since the birds used in these experiments were given only one feeding of infected slugs, it is apparent that all the tapeworms were of the same age.

FACTORS GOVERNING SEGMENT PRODUCTION

During the course of the work, wide variations were observed in the daily segment counts from the same bird, not only when the ration was changed but also during the intervals when the food consumption was decreased because of extremely hot weather or the presence of intercurrent disease. These observations suggested the several experiments described below.

The first was an experiment to test the effect of starvation for one day on the daily segment counts. Preliminary segment counts were done on two birds for five days. They were starved during the sixth day and were fed as usual on the seventh. The data in Table 3 show that there was a marked decrease in the number of segments passed during the starvation period. The segment count dropped still further during the two succeeding days in spite of the fact that the birds were being fed as usual. The subsequent counts gradually increased until the normal level was reached.

The second experiment was done to determine whether the growth of *D. proglottina,* as determined by segment counts, would be affected by feeding the host a ration containing little or no food value.

Bird 395 was isolated and the total number of segments discharged each day determined. The segment counts on seven successive days were 46, 49, 34, 47, 45, and 41. On the morning of the 8th day, the regular feed was withdrawn and moistened soft pine sawdust was force-fed. This was done for four days. The segment counts on these days were 35, 1,

[1] The largest number of segments passed in 24 hours in no instance exceeded the number of scoleces found post mortem by more than eighteen. It is quite probable that those scoleces were overlooked when the count was made.

TABLE 3.—*The number of segments of* D. proglottina *discharged by parasitized birds during 24-hour periods before and after a period of starvation*

	July															August	
	12	18	19	20	21	Food withheld for 24 hours*	22	23	24	25	26	27	28	29	30	31	1	2
Bird 978	267	335	318	158	431		82	47	34	52	77	77	114	223	271	222	213	349
Bird 990	178	200	202	172	143		78	39	30	80	166	174	220					

* Beginning with morning of July 22; ending with morning of July 23.

0 and 0 respectively. On the 12th day regular feed was given again. The segment counts on the following successive days were 0, 0, 0, 5, 10, 41, 32, and 69.

Since the sawdust might have contained substances that affected the tapeworms adversely, another experiment was done in which bran and ground cellophane were mixed in the mash in increasing amounts. The data in Table 4 indicate that as the bran content of the ration increased,

TABLE 4.—*The relationship of the daily segment discharge of birds parasitized with* D. proglottina *to the nutritive value of the feed*

Number days on experiment	Bird 998	Bird 982	Remarks
1	454	227	
2	503	252	
3	467	251	Ration: 6% Dry Skim Milk
4	427	302	94% Mash
5	482	252	
6	446		
7	238	209	
8	309	180	
11	342	150	Ration: 10% Bran
12	344	135	90% Mash
14	277	71	
18	246	66	
25	240	141	
31	305	72	
32	280	77	Ration: 47% Bran
33	178	111	53% Mash
34	169	78	
35	308	77	
36	113	82	
37	150	119	Ration: 57% Bran
38	148	96	43% Mash
39	290	137	
40	305	93	50% Bran Ration: 38% Mash 12% Cellophane
41	42	125	30% Bran
42	5	78	Ration: 24% Mash
43	1	20	46% Cellophane
44	0	4	
45	4	0	
46	69	18	
47	90	45	
48	153	38	Ration: 6% Dry Skim Milk
49	160	87	94% Mash
50	388	80	
51	328	168	
52	403	180	
53	456		

the segment counts decreased to levels considerably below the normal. When the cellophane was added, the segment counts took a precipitous drop to zero. The resumption of feeding the normal ration resulted in a steady rise in the number of segments discharged. It should be noted that both birds ate freely of the various rations offered. It was also found that as the nutritive value of the feed decreased, the amount of feces passed by the birds greatly increased.

DISCUSSION

These data justify the conclusion that each worm (*D. proglottina*) produces a gravid segment not oftener than once every 24 hours. It follows, then, that there are at least as many worms in the intestines of the host as the largest number of segments discharged in any 24 hour period. Under some conditions there may be more scoleces present than the number of segments eliminated in one day.

Of interest is the fact brought out by our observation, that the growth and development of all tapeworms of the same age are not uniform. While some were producing gravid segments almost every day, others contained very short strobilae and produced no gravid segments. This may be the explanation for Wetzel's results when he found that birds fed large numbers of isolated cysticercoids discharged very few gravid segments. This may also be the true explanation for the finding of tapeworms of various sizes in one intestine by Kalkus (1928), which was interpreted by him to be due to the difference in the age of those worms.

It has long been assumed that growth of tapeworms is dependent on the presence of absorbable nutrients in the intestinal canal of the host. Proof of this assumption has never been presented elsewhere, so far as the writer can ascertain. That a certain minimum of nutrients must be present for segment production was shown in experiments in which sawdust, and mixtures of bran and cellophane were used to decrease the nutritive value of the feed. Even though, in the latter experiment, 24 per cent of the ration consisted of the regular mash, segment production practically ceased

The cyclic discharge of segments in which the peak takes place a short time before dark, no matter what the season of the year may be, is of considerable practical significance from the standpoint of the transmission of the parasite. It is obvious that segments, that have been freshly passed and that have not been exposed to the destructive influence of the midday sun, are more likely to infect slugs, which are nocturnal in habit, than those that had been passed earlier in the day.

SUMMARY

A new method for counting the small tapeworm (*D. proglottina*) in the intestine of chickens is described.

The daily periodicity of segment discharge noted by Wetzel was confirmed. During the summer months the peak of segment discharge occurred between 5:00 PM and 9:00 AM. In the winter the peak was reached between 2:00 PM and 4:00 PM. This shift in the time of segment discharge is apparently dependent on the shortening of the days in the winter with the resultant effect that the feeding time of the birds is

altered. The time of greatest segment discharge can be altered at will by changing the feeding hours of the host.

The total number of segments discharged each day tended to remain at about the same level so long as feeding and other environmental influences were unchanged. Under optimum conditions, so far as the parasite is concerned, each worm is capable of shedding one ripe segment about every 24 hours.

Feeding the host on rations with little or no food value not only resulted in a marked decrease in segment production, but, in some instances, caused segment production to cease entirely.

The starvation of the host for 24 hours caused a decrease in the number of segments discharged during the following week, after which time the normal segment discharge was attained.

Not all tapeworms reach the same stage of development in the intestine even though they are of the same age.

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A SURVEY OF INTESTINAL PROTOZOA AMONG CHILDREN AND ADULTS IN LOS ANGELES

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A number of reports have been published from California which give the incidence of intestinal protozoan infection in hospital patients and in groups examined in medical practice who were suspected of having gastro-intestinal symptoms (see Barrow (1924), Kofoid (1926), Kessel and Mason (1930)). More recently, two reports have appeared from non-clinical groups, one by Johnstone, David and Reed (1933) on a group of prisoners from San Quentin and one by Iverson and Johnstone (1937) on food handlers from San Francisco.

The present report records the results of examination[2] of 2016 individuals, examined for purposes of survey alone, these persons exhibiting no gastro-intestinal symptoms for which they sought medical examination. They belong to three groups: (1), 585 adults seeking work as kitchen employees in the Los Angeles County Hospital; (2), 753 adult clinic patients seeking medical attention for conditions other than gastro-intestinal in nature; (3), 678 children from a Los Angeles City Juvenile Detention Home.

Six consecutive daily stools were examined from the first group of individuals, first by fresh iodine-eosin smear and second, by permanent iron-hematoxylin smear. Since it was possible to collect only one stool from the second and third groups, these were collected following a saline purge, a semi-solid or liquid stool, therefore, being examined by the same smear and staining methods as group one.

This second method of collecting stools for examination is in common use and Svensson (1935) compared its value with other methods. She concludes from the material she examined that 72 per cent of the actual infections of *Endamoeba histolytica* are detected by examination of six formed stools while 75 per cent are detected by examination of one loose stool following a purge. Kessel and Svensson (1924) estimated from their survey that examination of six formed stools detected approximately 90 per cent of the actual infections but Svensson (1935) has shown that the percentage so detected may vary with different groups, that the variation probably is correlated with the intensity of infection present in the

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[1] Assistance rendered by W. P. A. Project.

[2] With the technical assistance of Margaret Parrish and E. Burwell Goolden. Appreciation is extended to Dr. H. Covey and staff of the Juvenile Hall for cooperation in collecting specimens from the children.

TABLE 1.—*Intestinal protozoan survey—Los Angeles*

	Total examined	Positives	Per cent for all protozoa	*E. histolytica*		*E. coli*		*E. nana*		*I. butschlii*		*D. fragilis*		*Giardia*		*Chilomastix*		*Trichomonas*		*Enteromonas*	
				No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
KITCHEN EMPLOYEES—1934–37 Los Angeles County Hospital (Age Average 37)																					
Males	309	130	42	19	6	64	21	58	19	9	3	4	1	12	4	10	3	7	2	14	4
Females ..	276	118	43	7	3	66	24	55	20	10	4	6	2	13	5	27	10	7	3	10	4
Total ...	585	248	42	26	4	130	22	103	18	19	3	10	2	25	4	37	6	14	2	24	4
ADULT CLINIC GROUP (Age Average 52)																					
Males	336	72	21	9	3	40	12	20	6	4	1	3	1	5	1	9	3	3	1	3	1
Females ..	417	92	22	8	2	48	12	30	7	5	1	5	1	7	2	14	3	10	2	1	*
Total ..	753	164	22	17	2	88	12	50	7	9	1	8	1	12	2	23	3	13	2	4	*
Total Adults ..	1338	412	30.8	43	3.2	218	16.3	153	11.4	28	2.1	18	1.3	37	2.8	60	4.4	27	2.0	28	2.1
JUVENILE GROUP (Ages 10–15)																					
Males	460	190	41	19	4	102	22	55	12	24	5	10	2	45	10	9	2	4	1	4	1
Females ..	218	77	35	5	2	48	22	24	11	8	4	7	3	15	7	1	*	1	*	1	*
Total ..	678	267	39	24	3	150	22	79	11	32	5	17	3	60	9	10	1	5	1	5	1
Grand Total ..	2016	679	33.7	67	3.3	368	18.3	222	11.0	60	3.0	35	1.7	97	4.8	70	3.5	32	1.5	33	1.6

* Less than 1 per cent.

group in question and that the 90 per cent estimate mentioned above is too high for most surveys.

One may be assured, however, that the figures obtained by the two methods used in the present survey give a representative report of the incidence of intestinal protozoa present in the groups examined, though the actual incidence would be from 10 to 25 per cent higher if corrections were made.

The table shows the numbers and percentages obtained for the different species of protozoa in the three groups of people examined, each group having been divided into males and females.

The following points of interest may be mentioned for comparison with other surveys.

1. The incidence of infection is higher in the middle age group than in the older adult group or in the children.

2. The incidence for all protozoa is approximately the same in males and females in the two adult groups while it is higher in the males than the females in the juvenile group.

3. The incidence of *E. histolytica* is higher in the males than females in all groups.

4. The incidence of *Giardia* is markedly higher in the juvenile group than in the adults.

5. The incidence of *E. histolytica* infections determined by this survey is lower than those reported in California where special clinic groups were examined. It is also lower than the report of the San Quentin group but slightly higher than the report of Iverson and Johnstone (1937) in their food-handler survey.

6. Since an insufficient number of Mexican, Oriental and colored individuals occurred in this survey to warrant a statistical comparison of the incidence of infections in the different races, the tabulated results are not included in the chart. However, of 345 individuals examined who did not belong to the white race 31 per cent showed the presence of intestinal protozoa and 4.3 per cent were positive for *E. histolytica.*

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ECHINOCOCCUS INFECTION IN LOUISIANA

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Summarizing the reports on hydatid disease in the United States, Magath (1937) concludes: "A native citizen of the United States, who has never left his country, so rarely has hydatid disease that such an event will occur less often than once in five years." Thus the report of such a case of echinococcus cyst found in a native at necropsy may be justified.

The patient, G. St., 72 years of age, male, white, born in Minnesota, came to New Orleans, La., when eight years old and never left this city. Neither he nor his wife remembers any disease during all these years and he never left his work for sickness. He was out of work only the last two years because of his age. In the last six months he noticed a swelling of his abdomen. Admitted to the Charity Hospital of Louisiana on June 17, 1937, his clinical picture was that of liver cirrhosis with ascites. The Wassermann reaction was negative, the X-ray plate of thorax and abdomen showed marked distension of the abdomen and increase in lung markings. The patient died on July 16, 1937, and the necropsy (Charity Hospital, New Orleans, La., A–37–712 T.) confirmed the clinical diagnosis: Laennec's atrophic cirrhosis of the liver. The liver was small, shrunken, atrophic, very rough and irregular, and nodular with increased consistency. Microscopically much of the liver parenchyma was replaced by fibrous connective tissue.

In addition to this cirrhosis, on section the dome of the liver on the right side was the seat of a spherical encapsulated cyst measuring 4 cm in diameter and filled with thick, creamy, yellow, pasty material. The capsule of this cyst was very firm, hard and calcified, and about 1 mm in thickness. The microscopic examination of a decalcified section of the cyst wall showed the laminated chitin layers indicative of echinococcus cyst. The autopsy was performed by Dr. H. J. Schattenberg (Department of Pathology, Tulane University, New Orleans, La.).[1]

Since such an echinococcus cyst can develop only after ingestion of eggs of the echinococcus adult, which lives in the intestine of dogs, this patient must have come in contact with dogs infected with this tapeworm. No pet dogs were ever kept in the family of this patient. He was, however, employed at a railroad depot where he had to feed and to take care of the animals sent by express. Among these animals there were often

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[1] My thanks are extended to Dr. Rigney D'Aunoy, Director of Laboratories, Charity Hospital of Louisiana, New Orleans, for access to this and the other autopsy reports cited in this paper.

dogs and it seems plausible to assume that the infection may have been acquired from one of these dogs. The calcification of the cyst wall indicates the infection occurred 10 or more years ago.

The rarity of hydatid cysts found in human beings in the United States would suggest that echinococcus in dogs is very rare, too. No such survey of dogs seems to have been made, either in Louisiana or elsewhere in the United States, and Magath (1937) was able to find only one record, that by Curtice, who, in 1892, found a natural infection of *Echinococcus granulosus* in a dog killed at the pound in Washington, D. C. Although apparently not recorded, another natural infection of a dog was found in 1922 in Georgia by field workers of Dr. B. H. Ransom, at that time chief of the Zoological Division of the U. S. Bureau of Animal Industry. Specimens of this material sent by Dr. Ransom are in the helminthological collection of Dr. E. C. Faust. The conclusion from these two cases, however, that echinococcus occurs only exceptionally in the United States, is disproved by the frequent finding of hydatid cysts in sheep, cattle and hogs. The records of the meat inspection for the years 1898–1899 (Salmon, 1901) show, for example, an incidence of echinococcosis of 5–20 per cent, in hogs slaughtered in Louisiana, although this incidence has fallen since then (Morris, 1927). Riley (1933) explains the low incidence in the definitive host and man and the high incidence in these intermediate hosts by the possibility that wild carnivores may play the rôle of the definitive host. Until more data on the incidence of *Echinococcus granulosus* in dogs and possible other hosts are known, this paradoxical situation remains unsolved.

In Louisiana, the first case of hydatid disease was reported by Ogden in 1882, in a woman, a foreigner (Osler, 1882), the second by Smith (1928), in a woman, a native of Arkansas who might have contracted the disease there. The third case, recently published by Miller and Collins (1937), is that of a negress, native of Louisiana, who never left the state. In addition to these recorded cases there were found in New Orleans in 1906 a case of echinococcus cyst of the liver in a white woman, 36 years of age, a native of Louisiana, who resided here her entire life; in 1913 a case of echinococcus cyst of the liver in a white woman, 64 years of age, a native of Mississippi; in 1932 a case of echinococcus cyst of the liver in a white male, 38 years of age, native of Idaho, which he left when 11 years old. He then came to New Orleans, La., and spent ten years as a fisherman and oysterman in the Louisiana marshes. He was previously operated upon, in 1923, in the Walter Reed General Hospital, Washington, D. C., with the drainage of the cyst. In 1937 a calcified echinococcus cyst of the breast was found in a colored woman, 53 years of age, a native of Louisiana, a farmer's wife. Thus the number of cases in the United States to date totals 391; 385 summarized by Magath

(1937), one by Miller and Collins (1937), four unreported instances in Louisiana and the subject of the present report. Eight of them have occurred in Louisiana. Twenty-five were native born.

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TWO NEW MYXOSPORIDIA FROM TIDE POOL FISHES OF CALIFORNIA

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Specimens of the spotted kelpfish, *Gibbonsia elegans elegans* (Cooper), and the tide pool blenny, *Hypsoblennius gliberti* (Jordan), were collected at Santa Barbara, California, and examined for myxosporidia. Fourteen of the former and seventeen of the latter were obtained during the months October–January, and a one hundred per cent infection of the gall bladders was found. This high percentage is probably correlated with the habit of dwelling on the sea bottom in a restricted locality.

Since it was found that fixatives and stains in general tended to shrink the spores and give them an abnormal wrinkled appearance, almost all of the following descriptions are based on observations of living material. Kudo's (1930) method of using two coverslips and a depression slide was employed. Examination of living material was made without adding saline or other diluting agent since the fluid of the gall bladder was sufficient.

Schaudinn's, Bouin's and Champy's fixatives were used, followed by Heidenhain's iron hematoxylin and Delafield's hematoxylin. These stains were useful in bringing out the nuclei of trophozoites.

Leptotheca elegans n. sp.
(Figs. 1–7)

Specific diagnosis: Genus *Leptotheca.*

Trophozoite (figs. 1–2), rounded forms 20–26 microns in diameter, club-shaped forms up to 65 microns long. Ectoplasm thin; endoplasm very granular, often with large refractive globules (fig. 3). Pseudopodia small, delicate, clear and pointed; movement sluggish. Disporous (fig. 7).

Spore egg-shaped in polar view (fig. 4), breadth 17 microns, sutural diameter 9 microns; flattened with lateral swellings in lateral view (fig. 5). Lightly granular sporoplasm fills spore. Two polar capsules 3 by 2.2 microns. Suture line indistinct and divides spore into slightly unequal valves. Trinucleate spore occasionally present (fig. 6).

Host: Gibbonsia elegans elegans (Cooper).

Location: Gall bladder.

Locality: Tide pools of Santa Barbara, California.

Because of the peculiar shape of the spore (lateral view) there are no other species of the genus *Leptotheca* that can be considered closely similar to *L. elegans.*

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Ceratomyxa blennius n. sp.
(Figs. 8–12)

Specific diagnosis: Genus *Ceratomyxa.*

Trophozoite polymorphic but generally club-shaped (figs. 8–9); length up to 80 microns, width to 20 microns. Ectoplasm not distinct from granular endoplasm; pseudopodia needle-like, delicate, generally restricted to anterior end. Disporous (fig. 10). No movement observed.

Spore crescentic in polar view (fig. 11), oval in lateral view (fig. 12); valves equal with ends rounded. Breadth 22 microns, sutural diameter 7.3 microns. Two large, round polar capsules 3 microns in diameter. Sporoplasm lightly granular, fills equal valves; each valve nucleus with single karyosome.

Host: Hypsoblennius gliberti (Jordan).

Location: Gall bladder.

Locality: Tide pools of Santa Barbara, California.

This species is most closely related in appearance to *C. monospora* Davis (1917) and *C. arcuata* Thélohan (1892). It differs from the former in being three times as long (maximum) and only disporous. Its spores are curved in a polar view while those of *C. monospora* are curved in a lateral view. The valves are equal with rounded ends in the former instead of unequal with more pointed ends as in the latter. Also, in *C. blennius* the sporoplasm fills both valves while in *C. monospora* it does not fill both valves.

C. blennius differs from *C. arcuata* in that the pseudopodia are not always localized at the anterior end of the former, and the endoplasm is without large refractive fat globules. The length of the latter is only 40 microns. *C. blennius* appears stouter because its spores have a greater arch than those of *C. arcuata.*

Kudo's (1933) revised classification has been used. The two species of myxosporidia described in this paper, with those described by Jameson (1929 and 1931) are the only myxosporidia reported to date from the California coast.

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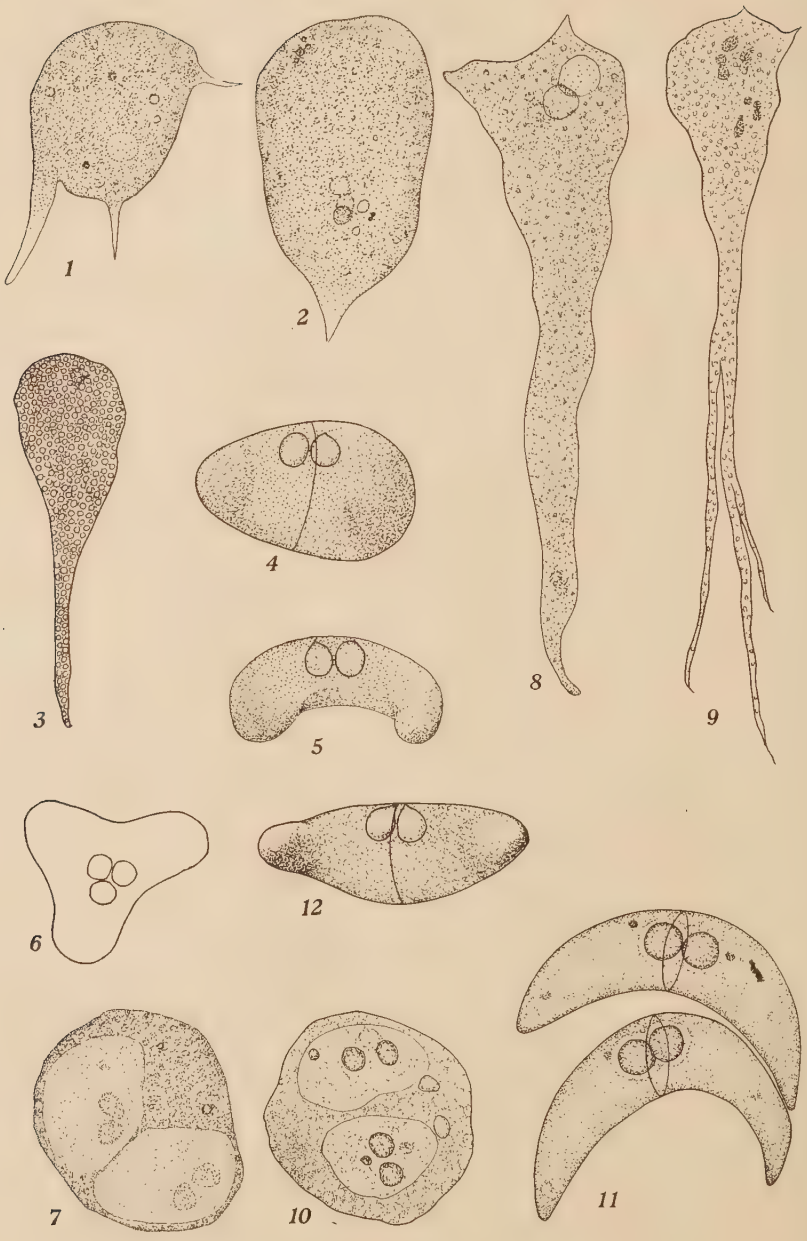
EXPLANATION OF PLATE, p. 444

All figures (present magnification × 1800) were drawn from living material with the aid of a camera lucida.

FIG. 1. Trophozoite of *Leptotheca elegans* with three pseudopodia.
FIG. 2. Same as above but with one pseudopodium.
FIG. 3. Trophozoite of *L. elegans* with large refractive globules.
FIG. 4. Polar view of spore of *L. elegans.*
FIG. 5. Lateral view of spore of *L. elegans.*
FIG. 6. Triangular spore occasionally seen with normal spores of *L. elegans.*
FIG. 7. Trophozoite of *L. elegans* with two developing spores.
FIG. 8. Trophozoite of *Ceratomyxa blennius.*
FIG. 9. Trophozoite of *C. blennius.*
FIG. 10. Trophozoite of *C. blennius* with two developing spores.
FIG. 11. Polar view of spore of *C. blennius* showing crescent appearance.
FIG. 12. Lateral view of spore of *C. blennius.*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PHYSIOLOGICAL OBSERVATIONS ON A LARVAL *EUSTRONGYLIDES* (NEMATODA)[1]

THEODOR VON BRAND[2]

The studies so far published on the metabolism of helminths deal with forms living in the intestinal tract or the bile ducts, and up to the present no information has been available concerning the metabolism of parasites living in the tissues proper. This is partly due to the difficulty of getting enough material for chemical analysis. The writer has recently had the opportunity, however, to obtain a sufficient quantity of the larval *Eustrongylides* described by Mueller (1934).

MATERIAL

The source of the helminthic material used in these observations was *Fundulus heteroclitus* from the Chesapeake Bay, heavily parasitized with these nematodes. One lot of 62 fishes examined quantitatively in September, 1937, showed 55 infected, yielding 157 parasites, an average of nearly 3 worms each. Individually, the fishes harbored from one to eight worms. In general larvae from the same host were approximately of the same size, although occasionally very small specimens were present with larger ones, indicating infections contracted at different times. The worms were almost exclusively found in fibrous capsules attached to the mesenteries, as Mueller described. In two instances, however, a worm was in a cyst located in the muscles of the body wall. Only the larger worms, weighing 30 to 130 mgm, were used in the present study.

The nematodes had no harmful effect on their host, as judged by the fact that parasitized fish could be kept for months in an aquarium. When the host did die the worms left the fibrous capsules and crawled around in the body cavity. It was observed frequently that they tried to escape from the dead body by burrowing through its tissues, commonly piercing the gill region, occasionally the abdominal muscles and sometimes even through the thick dorsal muscle layer.

SURVIVAL OF THE PARASITES IN VITRO

Three series of experiments were carried out in order to determine the survival of the worms in vitro. Of these, the third was conducted under sterile conditions.

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[1] A contribution from the Department of Helminthology, School of Hygiene and Public Health, Johns Hopkins University, and Barat College of the Sacred Heart, Lake Forest, Ill.

[2] The author is greatly indebted to Dr. W. W. Cort for having made possible the performance of these experiments.

In Series I the survival of worms starving in 1 per cent saline was studied at different temperatures. Six experiments with 8 worms were carried out at 37° C. The worms remained alive from 3 to 19 days with an average survival of 10 days. The corresponding data for 5 experiments with 7 worms conducted at room temperature are 10 to 26 days with an average of 17 days. In a refrigerator, the mean temperature of which was about 4° C, 5 worms remained alive for 60 to 95 days with an average of 72 days. It can be seen from these data that the range of temperature, under which the worms can live, is large.

In Series II the worms were kept at 37° C on two nutritive media. On egg slants covered with Ringer's solution 5 worms lived from 10 to 17 days with an average of 15 days. On this medium the worms were very active for the first 4 to 5 days, after which they became more sluggish. They would burrow deep into the egg slants. In one instance it was observed that a worm about 10 cm in length required only an hour to burrow its full length into the coagulated egg.

On egg slants covered with Ringer's solution plus 2 per cent Lilly's liver extract 5 worms lived from 6 to 13 days, the average being 9 days. Because of pronounced bacterial growth, the supernatant fluid was renewed every day or every other day in all experiments of Series II. For the same reason all the worms living long enough were transferred every week to a new egg slant.

It was felt that bacterial activity might have interfered with the viability of the worms. Series III was therefore carried out under sterile conditions. Because of the difficulty of extracting the worms aseptically from the fishes only 4 experiments, all at 37° C, could be performed.[3] The first worm, kept on an egg slant covered with Ringer's solution, remained alive for 34 days. The other three worms each lived in a test tube containing 5 cc of dextrose-broth (13 gm mixture of bacto-beef extract, bacto-peptone, and bacto-dextrose in 1000 cc water). One died after 48 days. The other two were still alive and active after 59 days. After 82 days, the next occasion when an observation was possible, they had died. By this time most of the fluid had evaporated and the worms were in a gelatinous mass. This series demonstrates that in absence of bacteria the length of life in vitro was greatly increased. If an aseptic change of medium had been possible, the worms might have lived longer.

In all these experiments no indication of development was found; it was a mere survival.

CHEMICAL COMPOSITION OF THE WORMS

Some data concerning the chemical composition of the worms were secured from parasites taken from freshly caught fish. The following

[3] I am indebted to Dr. G. F. Otto, who observed the worms after I left Baltimore.

methods were used. Dry substance was determined by drying worms at 100° C until the weight remained constant, the inorganic substances by incineration of dry substance and the ether extract by extraction of pulverized dry substance in a Soxhlet apparatus. The glycogen was determined according to v. Brand's (1936) micro-modification of Pflueger's method.

The mean values and the extremes of these determinations are summarized in table 1.

TABLE 1.—*Chemical composition of larval* Eustrongylides

	Number of determinations	Substances in per cent of the fresh worms	
		Mean value	Extremes
Dry substance	4	25	22 – 28
Inorganic substances	2	1.1	1.0 – 1.2
Ether extract	2	1.1	1.1 – 1.2
Glycogen	14	6.9 ± 0.3	4.8 – 9.0

The general chemical composition resembles that of the adult *Ascaris,* the only other parasitic nematode from which quantitative data are available. The similarity is chiefly evidenced by the low fat and the very high glycogen content.

In addition to the chemical determination on the whole worms, morphological observations were made upon the distribution of the glycogen in the worm body. Some worms were fixed in Carnoy's solution and the sections stained with Best's carmin using Ehrlich's hematoxylin as counter stain. Control sections were stained in the same way after having been digested for one hour with filtered saliva at 37° C. Almost all the glycogen was found in two places, the muscle cells of the body wall and the epithelial cells of the intestine, with exception of the esophagus. The histological details corresponded in the muscle cells exactly to those known from *Ascaris* (von Kemnitz, 1912). Large amounts of glycogen were also found in this form in the plasma bulb of the muscle cell, whereas only little was seen in the region of the contractile elements proper. The epithelial cells of the intestine were full of glycogen; only the nucleus appeared blue in the otherwise deeply red cells. This deposition of apparently large amounts of glycogen in the intestinal cells is of interest, since in trematodes, for example, the intestine is glycogen-free (Ortner-Schoenbach, 1913), and in *Ascaris* its glycogen content seems to be very variable (von Kemnitz, 1912).

As stated previously (von Brand, 1937) the red color of the worms is due to hemoglobin. In addition to the observations already recorded, it may be emphasized that the worms do not lose their bright red color as long they are living. Even if kept for one or two months in a hemoglobin free medium at 37° C, they are still red. This substantiates further the view that the hemoglobin is a true constituent of the body

and that its presence is not accidentally connected with the feeding on host tissues containing blood.

THE GLYCOGEN METABOLISM IN VITRO

The investigations upon the metabolism of parasitic worms have shown that in all forms so far investigated the glycogen metabolism is predominant (see reviews by McCoy, 1935, von Brand, 1934b and Wardle, 1937). Since, also, in *Eustrongylides* much glycogen was found, it was decided to investigate some phases of its carbohydrate metabolism. The glycogen consumption in 24 hours during starvation at 37° C was determined. This temperature was chosen because it seems reasonable to suppose that the worms live under natural conditions in a warm-blooded host, after the intermediate stage in the fish. Moreover all previous investigations upon the metabolism of other helminths have been performed at this temperature and it was desirable to compare the different rates of glycogen consumption. The experiments were carried out both under aerobic and anaerobic conditions. In the former case the worms were kept in open containers, in the latter a hydrogen stream was passed through the saline. In both cases the glycogen was determined in the individual experiment in half of the worms at the beginning and in the other half at the end of the 24 hour period. In most experiments after 24 hours had elapsed the saline was heated to the boiling point in order to remove all the carbon dioxide formed. After cooling the saline was titrated with n/10 NaOH, using litmus as indicator, in order to determine the amount of organic acids produced.

The results of the glycogen determinations are summarized in table 2.

TABLE 2.—*Glycogen consumption of starving* Eustrongylides *at 37° C.*

	Anaerobic		Aerobic	
	Beginning	After 24 hrs.	Beginning	After 24 hrs.
Number of determinations	6	6	5	5
Total number of worms	76	76	60	60
Total weight of worms, gm.	4.41	4.31	3.53	3.55
Glycogen in per cent	5.8 ± 0.4	4.9 ± 0.4	7.0 ± 0.6	6.7 ± 0.5
Glycogen consumption per 100 gm. in 24 hrs., gm. { mean	0.9 ± 0.2		0.3 ± 0.1	
Glycogen consumption per 100 gm. in 24 hrs., gm. { extremes	0.6 – 1.6		0.1 – 0.5	

The glycogen consumption of this species under anaerobic conditions approximates that found in other helminths under similar conditions: *Ascaris* 1.4 gm (von Brand, 1934a), *Fasciola* 2.8 gm (Weinland and von Brand, 1926), *Moniezia* 1.0 gm (von Brand, 1933). Comparable experiments under aerobic conditions have so far been published for *Ascaris* only (von Brand, 1934a) which consumes aerobically 1.2 gm of glycogen in 24 hours. The ratio between aerobically and anaerobically consumed glycogen for *Ascaris* is therefore 1.0: 1.3. *Eustrongy-*

lides in contrast, consumes aerobically only 0.3 gm against 0.9 gm, the ratio being 1 : 3. In free living worms, *Lumbricus, Halla* and *Spirographis,* a mean ratio of about 1 : 5 is found (Lesser, 1910, von Brand, 1927), whereas in the equally free living *Owenia* which, however, is characterized by an astonishing resistance against anaerobiosis, the ratio is 1.0 : 2.7 (von Brand, 1927).

The following reasons seem to be responsible for these differences. If a free living worm, like the earthworm, is kept under conditions providing it with enough oxygen, the metabolism is purely oxidative. It yields relatively large amounts of energy from the breakdown of a relatively small amount of carbohydrate. Under anaerobic conditions, on the other hand, a fermentation process goes on. A great part of the energy contained in the sugar molecule remains in the endproducts. Therefore, the earthworm under anaerobic conditions has to consume much more carbohydrate than under aerobic conditions in order to get a sufficient amount of energy. The ratio between aerobic and anaerobic glycogen consumption will necessarily be high. *Ascaris,* on the other hand, is adapted to anaerobic life, normally gaining its energy from fermentations. Even if it is supplied with oxygen a great part of the glycogen is utilized by the fermentation process. Only a small part is used in connection with the oxygen uptake and even that is perhaps not totally oxidized (von Brand, 1934a). This behavior accounts for the low aerobic/anaerobic glycogen ratio. As already mentioned an intermediate ratio is found in the free-living polychaete *Owenia,* but so far nothing is known about the details of its metabolism. It seems, however, possible that in such forms the first steps of physiological adaptation to a parasitic life, as viewed from a metabolic standpoint, may be found.

In so far as *Eustrongylides* is concerned, its metabolism resembles more that of a free-living form than that of a parasite such as *Ascaris.* In all experiments performed anaerobically on *Eustrongylides* relatively large amounts of organic acids were produced, averaging 30 cc n/10 acid for 100 gm worms in 24 hours. Under aerobic conditions no organic acids were found, indicating that under these conditions the carbohydrate was probably more or less totally oxidized. It is, however, possible that, with the direct titration method used, small amounts of excreted neutralized acids may have remained undetected. Such an assumption could account for the fact that the glycogen ratio found was between that of worms living purely oxidatively and of those living primarily anoxidatively. Experiments on the respiratory quotient, which would elucidate this point, are very desirable. The fact that the aerobic metabolism of our form resembles that of a free-living worm rather than an intestinal parasite, may be linked to the presence of its

large amounts of hemoglobin. This probably assists the worm in getting the oxygen needed.

The experiments described above, together with the observation that *Eustrongylides* cannot be kept in vitro longer than 48 to 72 hours under strictly anaerobic conditions, suggests that under natural conditions its metabolism may be primarily oxidative. Before this conclusion can be drawn definitely, however, it will be necessary to secure data concerning the oxygen tension prevailing in the fibrous capsules in which the worms live. It is reasonable to expect that it is not very high. When this point has been determined, it will then be necessary to investigate whether or not the respiratory mechanism of the worm is effective enough to provide it at this pressure with the maximum of oxygen. In other words, determinations are desirable concerning the limits of oxygen pressure in which the oxygen consumption remains constant.

THE GLYCOGEN METABOLISM IN VIVO

In order to study the glycogen relationships in vivo a batch of fishes was kept first for several weeks in the laboratory and regularly fed with fresh liver. Then the glycogen content of some of these fishes and of their parasites was determined. The remaining fishes were starved and similar glycogen determinations were performed at different intervals. The results of these experiments are summarized in table 3.

TABLE 3.—*Glycogen content of host and parasites during starvation of the former at room temperature*

	Glycogen in per cent of the fresh organisms			
	Number of fish	Mean glycogen content and (extremes)	Number of worms	Mean glycogen content and (extremes)
Beginning of starvation	6	0.48 ± 0.05 (0.32 – 0.67)	14	8.1 ± 0.5 (6.8 – 10.1)
After 14 days of starvation	6	0.48 ± 0.09 (0.15 – 0.72)	10	7.9 ± 0.6 (5.5 – 10.0)
After 41 days of starvation	6	0.36 ± 0.08 (0.10 – 0.72)	17	8.4 ± 0.3 (7.4 – 9.2)
After 65 days of starvation	6	0.23 ± 0.03 (0.12 – 0.35)	7	7.8 ± 0.7 (5.2 – 10.2)

As could be expected the glycogen content of the starving fishes decreases slowly during starvation. During 65 days they lost about half of their glycogen. In the parasites, on the other hand, we find only irregular fluctuations without statistical significance. It is obvious that they are able to keep their glycogen content constant even if the host starves for a long time. If we assume that the parasites live in the fish aerobically and that the Q_{10} of their life processes is 2, we can calculate from the figures found in the experiments concerning glycogen consumption in vitro for the 65 day period a glycogen consumption of about 4 gm per 100 gm worms. The worms would have lost about half of their

glycogen if they had no means of replenishing their stores. That they are able to do so, must be explained by the fact that they are tissue parasites. As such they are not dependent upon the food of the host, as many intestinal parasites are, but they can apparently always get sufficient food from the host tissue.

SUMMARY

1. *Eustrongylides* can be kept in vitro in the temperature range of 4 to 37° C for long periods.

2. It survives longer if kept in sterile surroundings.

3. The chemical composition is chiefly characterized by the low fat and high glycogen content.

4. The hemoglobin found in the body fluid is apparently a true constituent of the body.

5. Quantitative determinations of glycogen consumption in vitro were performed under aerobic and anaerobic conditions. The results indicate that the metabolism of this species resembles that of free living forms rather than that of intestinal parasites.

6. The worms are able to keep their glycogen constant even if the host starves for 65 days.

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SPECIES DIFFERENTIATION IN THE COCCIDIA FROM THE DOMESTIC SHEEP[1]

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INTRODUCTION

The literature on coccidia and coccidiosis in sheep was reviewed in detail by Thomson and Hall (1931), who called attention to the difficulty of determining whether the oöcysts in the feces represent five distinct species, as described in the literature, or whether these oöcysts were simply variants of a single species. On the basis of oöcyst studies Balozet (1932) concluded that there are five valid species in sheep, and he redefined *Eimeria parva, E. nina-kohl-yakimovi, E. faurei, E. arloingi,* and *E. intricata.* Balozet considered the colorless, spherical oöcysts described as *E. galouzoi* by Yakimov and Rastegaeva (1930) to be spherical variants of *E. nina-kohl-yakimovi.* Becker (1934) followed Balozet in recognizing five valid species, and suggested that further study is needed to establish the identity of *E. aemula,* described by Yakimov (1931).

The present survey of the coccidia from domestic sheep was undertaken to determine the validity of identifications based on the morphology of the oöcyst and, in the event this proved to be feasible, to ascertain the species infecting sheep in the United States. The results obtained indicate that the unsporulated oöcyst is a reliable criterion for diagnosis, that the five species recognized by Balozet are valid, and that *E. galouzoi* and *E. aemula* are probably not valid. In addition to the valid species of coccidia heretofore recorded from ovine hosts, two new species of the genus *Eimeria* from sheep are described on the basis of oöcysts.

With relatively costly domestic animals such as sheep, cattle, and swine, it is impracticable for the diagnostician to conduct experimental infections on coccidia-free hosts for the purpose of confirming oöcyst identification. In view of this difficulty it is believed that detailed descriptions and supplementary figures of the unsporulated oöcyst constitute the most practical form of identification now available for the determination of the species of coccidia of sheep.

MATERIALS AND METHODS

The material consisted of cecal contents from 70 slaughtered lambs, 6 to 8 months old, and fecal specimens from 30 sheep of various ages,

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[1] The work reported in this paper was done at the field station of the Zoological Division, National Agricultural Research Center, Beltsville, Md.

making a total of 100 samples. The cecal samples were taken from lambs killed at the abattoir of the Animal Husbandry Division of the Bureau of Animal Industry, Beltsville, Maryland; 40 were from a shipment of lambs from Wyoming, 18 from lambs from Idaho, and 12 from lambs raised by the Animal Husbandry Division at Beltsville. The imported animals were killed immediately upon arrival, without having had access to local pastures, and their coccidial fauna represents, therefore, infection acquired before arrival, and probably before shipment. Fecal specimens from living hosts were obtained from 29 sheep of the flock belonging to the Zoological Division. One fecal sample was from the New York State Veterinary College at Cornell University, Ithaca, New York. In all, 18 fecal samples were from sheep originating in Idaho, 41 from Maryland, 1 from New York, and 40 from Wyoming.

Both cecal and fecal specimens were subjected to the same procedure. Small representative quantities of feces were thoroughly crushed in a mortar, mixed into a thin paste with cold water, washed with more water through a sieve having 32 meshes to the linear inch in order to remove coarse debris, and set aside in tall glass jars to settle. After settling for 2 or 3 hours, the supernatant fluid was poured off, and 1 cc lots of the remaining sediment were mixed with 14 cc of 35 per cent cane sugar solution in 15 cc centrifuge tubes, which were racked for one to several hours for flotation of oöcysts.

After flotation the central portions of the surface films of the tubes were removed to glass slides by means of a small wire loop, and examined for oöcysts under the low power of the microscope. Records were kept of kinds and relative numbers of oöcysts in each sample. Sediments containing large numbers or rare kinds of oöcysts were numbered and stored in water at slightly above freezing until needed for further study.

To obtain material for measuring and study, single loopfuls of surface material containing oöcysts were mounted and observed under the 4 mm and oil immersion objectives, care being taken to have enough fluid on the slide to prevent crushing and distortion. In studying details of oöcyst morphology, small amounts of normal saline saturated with eosin and 5 per cent potassium iodide in normal saline saturated with iodine, were mixed with loopfuls of the material before mounting. The wall and internal structures were emphasized by the penetration of the iodine and eosin. The sugar solution used for flotation was found to have an index of refraction satisfactory for accurate observation.

Standard conditions for determination of sporulation times were obtained by transferring debris-free loopfuls of flotation fluid containing oöcysts into the cylindrical pits of microculture slides, adding a shallow layer of water of uniform depth, and placing the slides to incubate at room temperature inside a moist chamber. Daily observations were

made to record the progress of sporulation. By centrifuging to hasten both sedimentation and flotation, oöcysts were obtained for sporulation tests only 2 or 3 hours after discharge from the host. This standard method permitted comparison of sporulation periods of oöcysts of different species, whether in the same or different fecal specimens.

KINDS OF COCCIDIAL INFECTION IN SHEEP

Analysis of the types of oöcysts in 100 fecal samples showed that mixed infections predominated (Table I). There were 4 negative specimens, 34 pure infections, and 62 mixed infections with two to four species in each case. Oöcysts of *E. arloingi* were found in pure infection in 28 samples, those of *E. parva* were found in 4 samples, and those of *E. nina-kohl-yakimovi* and *E. granulosa* in 1 sample each. Of the mixed infections the combination of oöcysts of *E. parva* and *E. arloingi* occurred 21 times; other random combinations of two to four types of oöcysts in a sample occurred less frequently.

Strong supporting evidence that a particular type of oöcyst represents a separate species consists of finding typical oöcysts of the kind in question in samples having no oöcysts of closely related species. In six of the ten samples containing oöcysts of *E. pallida,* the latter were associated with those of *E. parva,* the most closely related species, while in the remaining four samples there were no oöcysts of *E. parva.* Likewise, in one out of eleven samples in which oöcysts of *E. granulosa* were found there was no *E. arloingi,* the remaining 10 samples containing both species.

This survey brings out the fact that coccidial infection is the rule with normal sheep. The fact that 96 per cent of the fecal samples taken at random from apparently healthy sheep contained oöcysts, suggests that probably most sheep are carriers of coccidial infection at some time of their lives.

THE UNSPORULATED OÖCYST

Usual descriptions of coccidia based on the kind of oöcyst include such features in the sporulated oöcyst as oöcystic and sporocystic residual bodies, and morphology and dimensions of the sporocyst and sporozoites. There are no oöcystic residual bodies in oöcysts from sheep, and the presence or absence of sporocystic residual bodies is difficult to determine. Measurements of sporocysts and sporozoites have no specific value, since their size depends upon the size of the original sporont and oöcyst. For these reasons stress is placed in this paper upon the unsporulated oöcyst in which the protoplasmic mass or sporont is spherical in shape. At this stage the oöcyst is most typical in size, shape, color, and morphology.

In size, oöcysts from sheep feces are small (*E. pallida, E. parva, E.*

TABLE 1.—Eimeria *species represented by oöcysts found in fecal samples from 100 domestic sheep, showing the frequency of negative samples, pure infections with a single species and combinations of two or more species*

Negative								
	pallida	*parva*			*arloingi*			4
	pallida				*arloingi*			6
	pallida				*arloingi*	*granulosa*		2
	pallida				*arloingi*		*intricata*	1
		parva						1
		parva	*nina-kohl-yakimovi*		*arloingi*			4
		parva		*faurei*				2
		parva		*faurei*	*arloingi*			1
		parva		*faurei*	*arloingi*		*intricata*	4
		parva			*arloingi*			1
		parva			*arloingi*	*granulosa*		21
		parva			*arloingi*		*intricata*	5
		parva	*nina-kohl-yakimovi*		*arloingi*			6
				faurei	*arloingi*			1
				faurei	*arloingi*	*granulosa*	*intricata*	4
					arloingi			1
					arloingi	*granulosa*		28
					arloingi		*intricata*	2
						granulosa		5
								1
4	10	50	3	11	90	10	14	100

Totals at the side represent the frequency of each kind of single and mixed infection based on oöcyst identification; totals at the bottom represent the relative occurrence of each species in 100 samples.

nina-kohl-yakimovi), medium-sized (*E. faurei, E. arloingi, E. granulosa*), or large (*E. intricata*).

The shape is stoutly ellipsoidal to spherical (*E. parva, E. nina-kohl-yakimovi*), narrowly ellipsoidal (*E. pallida, E. arloingi, E. intricata*), ovoid or hen's-egg-shaped (*E. faurei*), or urn-shaped or pyriform (*E. granulosa*).

They are colorless and crystalline (*E. parva*), colorless and pallid (*E. pallida*), delicate salmon pink to pale brownish-yellow (*E. nina-kohl-yakimovi, E. faurei*), distinctly yellowish-brown (*E. arloingi, E. granulosa*), or opaque and dark brown (*E. intricata*).

The transparent, pale yellow to yellowish-green external coat is present over the wall in all species. The wall is colorless to pale yellowish-green (*E. pallida, E. parva*), transparent and pale brownish-yellow to distinct yellowish-brown (*E. nina-kohl-yakimovi, E. faurei, E. arloingi, E. granulosa*), or opaque and dark brown (*E. intricata*); the oöcyst wall is thick and transversely striated (*E. intricata*), or thin and homogeneous (all other species). There are two dark refraction lines, one on each side of the wall, which give a "double-contoured" appearance (*E. parva*), or a single dark refraction line is present between the oöcyst membrane and inner surface of the wall (all other species).

A polar cap is absent (*E. pallida, E. parva, E. nina-kohl-yakimovi, E. faurei*); this cap varies from a flat operculum to a conspicuous rounded cone or crescent (*E. arloingi*), or is conspicuous and constant (*E. granulosa, E. intricata*).

The micropyle is probably always present, but may be inconspicuous or imperceptible (*E. pallida, E. parva, E. nina-kohl-yakimovi*), or it is obscured or covered by the polar cap (*E. arloingi, E. granulosa, E. intricata*), or clear and conspicuous (*E. faurei*).

The early spherical sporont is small and pale (*E. pallida*), small and crystalline (*E. parva*), medium-sized and with relatively pale granulation (*E. nina-kohl-yakimovi, E. faurei, E. arloingi*), relatively larger and more deeply and densely granular (*E. granulosa*), or large and conspicuous through opaque wall (*E. intricata*).

Sporulation time as an aid in identification is a misleading and treacherous criterion due to the extreme sensitivity of the oöcyst to varying conditions of temperature and oxygen tension. Standard conditions must be established before sporulation times are reliable. To insure standard conditions in this study freshly floated oöcysts were isolated into the depressions of culture slides, covered with shallow, uniform layers of cold tap water, and allowed to incubate in a moist chamber at room temperature. No chemicals of any kind were used, since the complete absence of putrefying debris, the uniform layer of water and the constant temperature equalized oxygen tension. Results of sporula-

tion tests widely spaced in time could be compared. Under these conditions oöcysts from sheep develop oval spores within 24 hours (*E. pallida,* smaller *E. parva*), between 24 and 48 hours (larger *E. parva, E. nina-kohl-yakimovi, E. faurei, E. arloingi*), or require at least 72 hours (*E. granulosa, E. intricata*).

In the writer's opinion a valid description of a coccidian based only on the oöcyst must include analysis of the features described here, with dimensions of the oöcysts based on measurements of at least 50 specimens from at least 5 hosts, in order to eliminate the possibility of describing variant strains of a known species as new. In addition, written descriptions should be supplemented by adequate figures.

The species of *Eimeria* from sheep may be differentiated with the aid of the following key:

KEY TO OÖCYSTS OF *Eimeria* IN SHEEP

1. Polar cap absent 2
   Polar cap present 5
2. Ellipsoidal to subspherical; transparent; colorless; oöcysts 12 to 22 μ long .. 3
   Ellipsoidal or egg-shaped; transparent; delicate salmon pink to pale brownish-yellow; oöcysts 20 to 33 μ long 4
3. Ellipsoidal; pallid, with single dark refraction line marking inner surface of wall ..... *E. pallida* n. sp.
   Ellipsoidal to subspherical; clear-cut, with two dark refraction lines, one on each side of wall ..... *E. parva* Kotlán, Mócsy and Vajda, 1929
4. Ellipsoidal; stout; micropyle inconspicuous or imperceptible.
   E. nina-kohl-yakimovi Yakimov and Rastegaeva, 1930
   Egg-shaped; micropyle conspicuous at narrow end.
   E. faurei Moussu and Marotel, 1901
5. Wall opaque, thick, transversely striated, dark brown; oöcysts 39 to 53 μ long ..... *E. intricata* Spiegl, 1925
   Wall transparent, thin, pale yellowish-brown; oöcysts 17 to 42 μ long 6
6. Typically shaped like broad-shouldered urn, or pyriform; cap a soft, gelatinous, flat to slightly convex truncated cone situated upon the broad end of the oöcyst; early sporont densely granular ..... *E. granulosa* n. sp.
   Typically ellipsoidal; cap a tough, firm, rounded cone or crescent; early sporont not densely granular ..... *E. arloingi* Marotel, 1905

DESCRIPTION OF SPECIES

Eimeria pallida n. sp.
(Figs. 1 and 2)

Unsporulated oöcysts: Oöcysts 12 to 20 μ (average 14.2 μ) long by 8 to 15 μ (average 10 μ) wide; 5 to 20 consecutive oöcysts measured from each of 9 host animals, dimensions being based on a total of 100. Shape ellipsoidal, with equal curvature at both ends; variation slight; body relatively narrow and elongated, being 0.58 to 0.83 (average 0.70) as wide as long. Micropyle imperceptible; occasionally oöcyst wall at one end appears slightly paler than at other, suggesting inconspicuous micropyle. No polar cap. Wall thin, homogeneous, pale yellow to yellowish-green, giving a fragile, pallid appearance to oöcyst. Single, dark refraction line between oöcyst membrane and inner surface of wall. Coat over oöcyst transparent, pale yellow, half as thick as wall. Early spherical sporont 6 to 10 μ (average 8 μ) in diameter, sparsely and irregularly granular, pale and inconspicuous. Under low power oöcysts of *E. pallida* are small, slender, ellipsoidal, colorless, delicate, pallid, and inconspicuous.

Sporulation time: At room temperature most of the oöcysts develop ovoid spores within 24 hours after isolation into clean water.

Relationships: The oöcysts of *E. pallida* are distinguished from those of *E. parva* by their pallid appearance, relatively more narrow form, and presence of only one dark contour line, this being at the inner surface of the wall.

Distribution and relative occurrence: Oöcysts were found in fecal samples of sheep as follows: 9 from Maryland and 1 from Wyoming, a total of 10 out of 100 samples examined. The oöcysts of this species were associated with oöcysts of *E. arloingi* 10 times, with *E. parva* 6 times, with *E. granulosa* once, and with *E. intricata* once. *E. pallida* was not found in pure infection.

The fact that there are two distinct kinds of small, colorless oöcysts from sheep became apparent early in this study. The clear-cut, crystalline, double-contoured oöcysts of *E. parva* were found in 50 of the 100 samples examined. In 10 out of the 100 samples a second kind of oöcyst was found, the latter being more narrow in shape, pale, inconspicuous, and colorless in appearance, and showing under oil immersion a single black refraction line on the inner surface of the wall. These oöcysts were constant in size and shape from host to host, and showed no intergradation with those of *E. parva.* In 6 of the 10 samples in which oöcysts of *E. pallida* occurred they were associated with those of *E. parva.* Kotlán, Mócsy, and Vajda (1929) stated that they found oöcysts of *E. parva* in 7 out of 11 hosts, usually in large numbers, indicating that these workers were dealing with the same kind of oöcysts which Balozet characterized as *E. parva,* and not with the pale oöcysts described here for *E. pallida.* Oöcysts of *E. pallida* were found in only 10 per cent of the samples, whereas *E. parva* was found in 50 per cent of the samples examined; they occurred in large numbers in only 2 of the 10 samples in which they were present.

Eimeria parva Kotlán, Mócsy and Vajda, 1929
(Fig. 3)

Unsporulated oöcysts: Oöcysts 12 to 22 μ (average 16.5 μ) long by 10 to 18 μ (average 14.1 μ) wide; 5 to 10 consecutive oöcysts measured from each of 25 host animals, dimensions being based on a total of 200. Shape ellipsoidal to subspherical, with equal curvature at both ends in ellipsoidal forms, the only variation being in relation of breadth to length; they are typically stout, being 0.68 to 1.00 (average 0.85) as broad as long. No perceptible micropyle; wall over one end slightly thinner and paler than other, suggesting inconspicuous micropyle. No polar cap. Wall thin, homogeneous, faintly yellow to yellowish-green, demarcated on each side by a heavy black refraction line, giving a characteristic "double-contoured" appearance; these are the only oöcysts from sheep regularly having such a double contour; they exhibit a marked tendency to crumple and cave in when left for a time in the concentrated brine or sugar solution used for flotation. External coat covering entire oöcyst is pale yellowish-green, and half as thick as the wall. Early spherical sporont 7 to 14 μ (average 10 μ) in diameter, crystalline, clear-cut, with refractile granules and globules in irregular network. Under low power oöcysts of *E. parva* are small, clear-cut, colorless, crystalline, elliptical to subspherical, and conspicuous.

Sporulation time: At room temperature many of the smaller oöcysts of this species form oval spores within 24 hours, the majority do so within 48 hours after isolation of freshly discharged oöcysts into clean water.

Relationships: The clear-cut and conspicuous appearance, the more rotund form, and the two black lines delimiting the wall sharply distinguish oöcysts of *E. parva* from those of *E. pallida,* the most closely related species.

Distribution and relative occurrence: Oöcysts were found in fecal samples from sheep as follows: 9 from Idaho, 27 from Maryland, and 14 from Wyoming; or a total of 50 out of 100 samples. The oöcysts of this species were associated with oöcysts of *E. pallida* 6 times, *E. faurei* 6 times, *E. arloingi* 45 times, *E. granulosa* 5 times, and *E. intricata* 7 times. *E. parva* was found in pure infection 4 times.

Kotlán, Mócsy, and Vajda (1929) described *E. parva* from oöcysts measuring 11.4 to 14.3 μ in length and 9.5 to 11.8 μ in breadth. The oöcysts that Balozet (1932) described for this species measured 13.5 to 19 μ (average 17.1 μ) in length and 11 to 14 μ (average 13.5 μ) in breadth. In the present study 200 consecutive oöcysts from 25 hosts had dimensions of 12 to 22 μ (average 16.5 μ) in length by 10 to 18 μ (average 14.1 μ) in breadth. Apparently Kotlán, Mócsy, and Vajda saw only oöcysts in the smaller range of the species.

There is a marked tendency for oöcysts from a given host individual in a given sample to have fairly uniform size, the mean of which may differ considerably from that of specimens from another individual. If oöcysts from only a few hosts are measured these variations may appear distinct and discontinuous, but gradually assume a normal distribution curve as the number of host animals from which oöcysts are measured is increased.

The oöcysts of *E. parva* are ellipsoidal to spherical in shape, varying between 0.68 and 1.00 in ratio of breadth to length. Oöcysts in the spherical range are rare, but fit the description given for those of *E. galouzoi* by Yakimov and Rastegaeva (1930). If *E. galouzoi* is to be accepted as a valid species, the oöcysts should be found in fecal samples having no typical *E. parva,* in several hosts, and in significant numbers.

Eimeria nina-kohl-yakimovi Yakimov and Rastegaeva, 1930
(Fig. 4)

Unsporulated oöcysts: Oöcysts 20 to 28 μ (average 23.1 μ) long by 15 to 22 μ (average 18.3 μ) wide; 25 to 40 consecutive oöcysts measured from each of 3 host animals, dimensions being based on a total of 100. Shape usually ellipsoidal, occasionally slightly ovoid; relatively stout, being 0.67 to 0.91 (average 0.79) as broad as long. Micropyle usually imperceptible, occasionally visible under bright light if oöcyst is tilted from its side. No polar cap. Wall thin, transparent, pale, almost imperceptibly brownish-yellow, but never as deeply tinted as in ordinary specimens of *E. arloingi;* wall thinner over micropyle end, where it is markedly double-contoured. Single dark refraction line present, marking interface between oöcyst membrane and inside surface of wall. External coat covering entire oöcyst, transparent, pale yellowish-green, about half as thick as wall. Early spherical sporont pale, 12 to 18 μ (average 15.1 μ) in diameter. Under low power oöcysts of *E. nina-kohl-yakimovi* are pale, delicate, stout, elliptical, capless, and faintly tinted.

Sporulation time: At room temperature most of the oöcysts of this species develop oval spores during the interval between 24 and 48 hours after isolation of freshly discharged oöcysts into clean water.

Relationships: These oöcysts are distinguished from the large oöcysts of *E. parva* by the faint brownish-yellow tint, larger mean size, thin and double-contoured

wall at micropyle end, and single heavy refraction line marking the inner surface of the wall.

Distribution and relative occurrence: Oöcysts were found in samples of sheep feces as follows: 1 from Maryland and 2 from Idaho; a total of 3 out of 100 samples contained this species. *E. nina-kohl-yakimovi* was found associated with oöcysts of *E. parva* and *E. arloingi* twice; found in pure infection once.

Yakimov and Rastegaeva (1930) described this species from oöcysts found in the goat. The oöcysts were stated to be ovoid or egg-shaped, capless and usually without micropyle, to have a distinctly double-contoured wall, and to measure 18.9 to 25.4 μ in length and 14.4 to 21 μ in breadth. The oöcysts that Balozet (1932) described for this species from both sheep and goats were regularly ovoid, had a thicker and more markedly double-contoured wall than those of *E. faurei,* were about the same color, and measured 16 to 27 μ (average 19.8 μ) long by 13 to 21 μ (average 16.5 μ) wide. The specimens found in the present study in 3 out of 100 sheep fit Balozet's description better than that of the original describers. The oöcysts in question are intermediate between the colorless, crystalline oöcysts of *E. parva* and the pale salmon pink to brownish-yellow ones of *E. faurei* in tint, the wall is never as distinctly double-contoured as in oöcysts of *E. parva,* and they measure 20 to 28 μ (average 23.1 μ) long by 15 to 22 μ (average 18.3 μ) wide.

Eimeria faurei Moussu and Marotel, 1901
(Fig. 6)

Unsporulated oöcysts: Oöcysts 25 to 33 μ (average 28.9 μ) long by 18 to 24 μ (average 21 μ) wide; 5 to 20 consecutive oöcysts measured from each of 10 host animals, dimensions being based on a total of 100. Shape characteristically like hen's egg, the broad end a segment of a circle, the narrow end formed by gradual attenuation from middle; variation slight; 0.63 to 0.80 (average 0.73) as broad as long. Micropyle a distinct, clear gap in wall at narrow end, 2 to 3 μ (average 2.5 μ) in diameter. No cap over micropyle. Wall transparent, pale brownish-yellow, discontinuous at narrow end to form micropylar opening; color slightly more intense toward micropyle; larger oöcysts in general more deeply tinted than smaller. Boundary between oöcyst membrane and inner surface of wall marked by a single heavy, black refraction line. External coat faint yellowish-green, about half as thick as wall. Early spherical sporont conspicuous, 13 to 20 μ (average 16 μ) in diameter. Under low power oöcysts of *E. faurei* are egg-shaped, capless, delicate salmon pink to pale yellowish-brown, and have a clear, conspicuous micropyle at narrow end.

Sporulation time: At room temperature the majority of the oöcysts of this species form oval spores during the interval between 24 and 48 hours after freshly discharged oöcysts are isolated into clean water.

Relationships: Oöcysts easily distinguished from all others from sheep by constant hen's egg shape, conspicuous micropyle at narrow end, and absence of cap over micropyle.

Distribution and relative occurrence: Oöcysts were found in fecal samples as follows: 5 from Idaho, 4 from Maryland, 1 from New York, and 1 from Wyoming, or a total of 11 out of 100 samples. The species under discussion was associated with oöcysts of *E. parva* 6 times, *E. arloingi* 10 times, *E. granulosa* once, and *E. intricata* twice; it was not found in pure infection.

Moussu and Marotel (1901) described oöcysts from sheep having a range of 18 to 42 μ in length and 18 to 30 μ in breadth, with variation in

shape between spherical and ovoid. At that time they suggested no specific name, but indicated that the parasites belonged to the genus *Coccidium*. Later, the same authors (1902) elaborated upon their description, suggesting the name *Coccidium faurei* for the coccidia from sheep. Placed by later workers in the genus *Eimeria*, all coccidia from sheep were for a time collectively designated as *E. faurei*. Considering the general nature of the description as given by Moussu and Marotel and the great range in size and shape, these authors were probably dealing with mixed infections in which oöcysts of *E. parva, E. faurei, E. arloingi* and perhaps others, were present. With Marotel's (1905) description of *E. arloingi* on the basis of capped, ellipsoidal oöcysts from the goat, and the description of *E. parva* by Kotlán, Mócsy, and Vajda, the name *E. faurei* has come to designate egg-shaped, transparent, brownish-yellow, capless oöcysts with a conspicuous micropyle at the narrow end, although the original description did not specifically define the limits of the species as now known.

Eimeria arloingi Marotel, 1905
(Fig. 7)

Unsporulated oöcysts: Oöcysts 17 to 42 μ (average 27 μ) long by 13 to 27 μ (average 18 μ) wide; 5 to 25 consecutive oöcysts measured from each of 32 host animals, dimensions being based on a total of 500; this species shows the greatest range in size of any species of coccidia from sheep. Shape usually ellipsoidal, with considerable variation; oöcysts in middle range most regularly ellipsoidal; asymmetrical forms frequent, with sides of unequal curvature or with one or the other end slightly attenuated; typically narrow and elongated, being 0.54 to 0.89 (average 0.67) as broad as long. The micropyle is a gap in the wall beneath the cap, 2 to 3 μ (average 2.5 μ) in diameter, and best observed in specimens from which cap has been dislodged. Cap typically present over micropyle, 3 to 8 μ (average 5 μ) wide and 0.2 to 2 μ (average 1.2 μ) high, varying from an inconspicuous, flat operculum in smaller oöcysts to a prominent, transparent, pale yellow to yellowish-green rounded cone or crescent in medium-sized and large oöcysts. This cap is a definite, tough, lid-like structure, and easily dislodged; it is often observed hanging by a narrow edge from one side of the micropyle end, and often missing. Wall transparent, uniform in thickness, discontinuous beneath cap to form micropyle; varying in color from faint brownish-yellow to distinct yellowish-brown, usually increasing in intensity toward micropyle; color faint in smaller oöcysts, which often appear almost colorless, gradually increasing in intensity as size increases. Single heavy, black contour line present between oöcyst membrane and inner surface of wall. External coat colorless to faint yellowish-green, transparent, half as thick as wall. Early spherical sporont pale, 11 to 18 μ (average 14 μ) in diameter. Under low power oöcysts of *E. arloingi* are elongated, narrow, ellipsoidal, pale yellowish-brown, and have a more or less prominent cap over the micropyle.

Sporulation time: At room temperature the majority of the oöcysts of *E. arloingi* develop ovoid spores during the interval between 24 and 48 hours after the freshly discharged oöcysts are isolated into clean water.

Relationships: Oöcysts of this species having a conspicuous cap are distinguished from those of *E. granulosa*, the most closely related species, by their typically ellipsoidal outline, nature and shape of the cap, relatively pallid protoplasm in the spherical sporont, and shorter sporulation time.

Distribution and relative occurrence: Oöcysts were found in fecal samples as follows: 11 from Idaho, 40 from Maryland, 1 from New York, and 38 from Wyo-

ming; a total of 90 out of 100 samples examined were positive for this species. This species was associated with oöcysts of *E. pallida* 10 times, *E. parva* 45 times, *E. faurei* 10 times, *E. granulosa,* 9 times, and *E. intricata* 14 times; found in pure infection 29 times.

In his original description of *E. arloingi,* based on oöcysts from the goat, Marotel (1905) failed to note the wide variability which is a striking feature of the species, and merely stated that the oöcysts were ellipsoidal, possessed a polar cap, and measured 22 to 27 μ in length and 16 to 18 μ in width. Several observers have since seen oöcysts of this species in the feces of sheep. Balozet (1932) stated that the cap over the micropyle is sometimes flat, sometimes distinct and button-like, and that oöcysts are 25 to 38 μ (average 30.8 μ) long by 17 to 25 μ (average 21.4 μ) wide. Data gathered from observations and measurements of 500 oöcysts from 32 sheep in the present study indicate even greater variation. There is perfect intergradation in all features from small, stout, apparently capless, almost colorless oöcysts to large, elongated, conspicuously capped, yellowish-brown oöcysts, and a range of 17 to 42 μ in length and 13 to 27 μ in breadth. Marotel described only oöcysts in the medium range having well-developed polar caps.

Yakimov (1931) described *E. aemula* from sheep on the basis of ellipsoidal, often slightly egg-shaped oöcysts, having a distinct micropyle and no cap, measuring 20.4 to 36 μ in length and 17 to 25.5 μ in width, and having a ratio of breadth to length of 0.60 to 0.92. This general description might fit oöcysts of *E. arloingi* having poorly developed polar caps, or from which the cap has been dislodged, a rather frequent occurrence. The writer was unable to find any consistent type of oöcyst answering the description of *E. aemula.*

Eimeria granulosa n. sp.
(Figs. 8 and 9)

Unsporulated oöcysts: Oöcysts 22 to 35 μ (average 29.4 μ) long by 17 to 25 μ (average 20.9 μ) wide; 5 to 15 consecutive oöcysts measured from each of 9 host animals, dimensions being based on a total of 100. Shape usually like that of a stout, broad-shouldered urn, or pyriform, with cap on broad end; greatest transdiameter two-fifths of total length from micropyle end; considerable variation; typical shape most frequent in medium- and large-sized oöcysts, smaller ones often bluntly ellipsoidal; slightly asymmetrical shapes frequent in entire range; 0.59 to 0.82 (average 0.71) as broad as long. Micropyle a gap in wall beneath cap, 3 to 5 μ (average 3.7 μ) in diameter. Cap over micropyle prominent, 5 to 10 μ (average 7.5 μ) wide and 1 to 2.5 μ (average 1.7 μ) high; having the form of a broad truncated cone with flat or slightly convex top and a consistency of soaked gelatin as shown by tendency to crush and fray in similar ragged manner, occupying a relatively flat surface over micropyle; it is easily dislodged and often partly detached or missing. Wall transparent, pale brownish-yellow to yellowish-brown, color increasing in intensity toward micropyle; color faint and almost imperceptible in smallest oöcysts, increasing in intensity with size of oöcyst. Boundary between oöcyst membrane and inner surface of wall marked by single heavy, black refraction line. There is a marked tendency for oöcyst membrane to pull away from wall, or cave in beneath micropyle, after remaining for a time in the concentrated sugar solution

used for flotation. External coat transparent, pale yellow to yellowish-green, half as thick as wall, continuous over micropyle beneath cap. Early spherical sporont 14 to 23 μ (average 16.5 μ) in diameter, characteristically densely and uniformly granular, appearing distinctly darker and more conspicuous than in oöcysts of *E. arloingi,* and suggesting the specific name *granulosa.* Under low power oöcysts of *E. granulosa* are pyriform or urn-shaped, pale yellowish-brown, and have a broad, flat polar cap and a characteristic, large granular sporont.

Sporulation time: At room temperature the majority of the oöcysts require 72 to 96 hours for the development of oval spores after freshly discharged oöcysts are isolated into clean water.

Relationships: Oöcysts of this species are distinguished from those of *E. arloingi* by having bulging shoulders to give an urn-shaped appearance, flat cap of gelatinous consistency, densely granular early spherical sporont, and longer sporulation time.

Distribution and relative occurrence: Oöcysts were found in fecal samples as follows: 9 from Maryland and 1 from New York; a total of 10 out of 100 samples examined contained this species. The oöcysts under consideration were associated with those of *E. pallida* once, *E. parva* 5 times, *E. faurei* once, *E. arloingi* 9 times, and *E. intricata* once; not found in pure infection.

Eimeria intricata Spiegl, 1925

(Fig. 5)

Unsporulated oöcysts: Oöcysts 39 to 53 μ (average 47 μ) long by 27 to 34 μ (average 32 μ) wide; 5 to 20 consecutive oöcysts measured from each of 5 host animals, dimensions being based on a total of 50; oöcysts larger than those of any coccidia thus far reported from sheep. Shape ellipsoidal, with slight variation; relatively slender, being 0.57 to 0.80 (average 0.68) as broad as long. Micropyle a wide gap in wall beneath cap, 6 to 10 μ (average 8 μ) in diameter. Cap over micropyle prominent, 6 to 11 μ (average 9 μ) wide and 1 to 3 μ (average 2 μ) high, transparent, colorless to yellowish-green, crescent-shaped, lid-like, detachable. Wall characteristically opaque, transversely striated, yellowish-brown to dark brown, and thickened to 2 to 3 μ (average 2.5 μ); structurally two-layered, internal layer being twice as thick, more intensely colored, and more distinctly striated than outer; outer surface of external layer irregularly corrugated, giving mottled appearance to wall when observed from above. Single dark refraction line present between oöcyst membrane and inner surface of wall. External coat thin, pale yellow to yellowish-green, and continuous over micropyle beneath cap. Early spherical sporont 19 to 26 μ (average 23 μ) in diameter, visible through opaque wall. Under low power oöcysts of *E. intricata* are large, opaque, dark brown, ellipsoidal, and have a thick, striated wall and a wide, crescent-shaped, transparent polar cap.

Sporulation time: At room temperature most of the oöcysts of *E. intricata* require from 72 to 120 hours to develop oval spores after freshly discharged oöcysts are isolated into clean water.

Relationships: The oöcysts of *E. intricata* are easily differentiated from all others from sheep by the thick, opaque, dark brown wall with transverse striations, large size, and transparent polar cap.

Distribution and relative occurrence: Oöcysts were found in fecal samples as follows: 7 from Maryland, 1 from New York, and 6 from Wyoming; a total of 14 out of 100 samples examined contained this species. The species in question was associated with oöcysts of *E. pallida* once, *E. parva* 7 times, *E. faurei* twice, *E. arloingi* 14 times, and *E. granulosa* once; it was not found in pure infection.

SUMMARY

Results of a survey of fecal samples from 100 sheep for coccidial oöcysts showed 96 per cent infection, 34 per cent being pure infections with a single species and 62 per cent being mixed infections with two to

four species each. Size, shape, color, sporulation time, and morphology of the unsporulated oöcyst were the criteria used for species identification. The oöcysts of *Eimeria parva, E. nina-kohl-yakimovi, E. faurei, E. arloingi,* and *E. intricata* are redescribed. Oöcysts conforming to the description of those of *E. galouzoi* were found in the spherical range of *E. parva,* and oöcysts similar to those described for *E. aemula* were observed among atypical and capless oöcysts of *E. arloingi.* Two new species, *E. pallida* and *E. granulosa,* are described from oöcysts found repeatedly in fecal samples.

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Explanation of Plate

All figures were drawn with the aid of a camera lucida. The unsporulated oöcysts had been discharged from the host for at least six hours before drawings were made to allow the protoplasm to assume spherical shape. Size relationships are preserved. The projected scale shows actual dimensions in microns.

Fig. 1. *Eimeria pallida.* Unsporulated oöcyst with spherical sporont.

Fig. 2. *E. pallida.* Sporulated oöcyst with four spores, each containing two sporozoites.

Fig. 3. *E. parva.* Unsporulated oöcyst with spherical sporont.

Fig. 4. *E. nina-kohl-yakimovi.* Unsporulated oöcyst with spherical sporont.

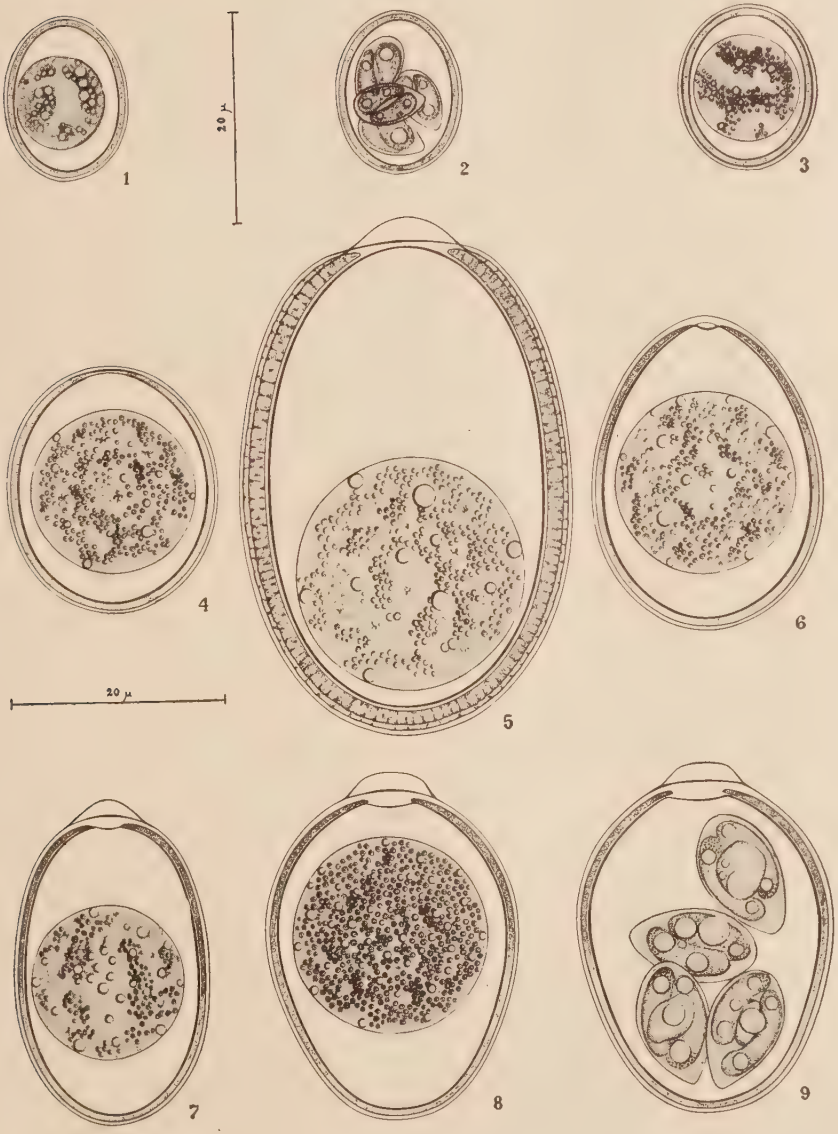
Fig. 5. *E. intricata.* Unsporulated oöcyst with spherical sporont.

Fig. 6. *E. faurei.* Unsporulated oöcyst with spherical sporont.

Fig. 7. *E. arloingi.* Unsporulated öocyst with spherical sporont.

Fig. 8. *E. granulosa.* Unsporulated oöcyst with spherical sporont, showing densely granular protoplasm.

Fig. 9. *E. granulosa.* Sporulated oöcyst with four spores, each containing two sporozoites and a residual body.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RESEARCH NOTES

A PRELIMINARY REPORT ON THE INFLUENCE OF HYDROGEN-ION CONCENTRATION UPON THE LONGEVITY OF *STRONGYLUS VULGARIS* (LOOSS, 1900) IN VITRO

In the course of preliminary attempts to culture adult *Strongylus vulgaris* in vitro, a relationship between the acidity of the media and the longevity of the worms was evident. No experiments on the influence of hydrogen-ion concentration on adult nematodes have been noted although McCoy (1930, Am. J. Hy. **11**: 413–448) has reported upon the effect of pH upon the eggs and larvae of hookworms, and Schmeleff (1929, Vestnik. Mikr. Epidemiol. **8**: 319–324) its effect upon ascarid eggs.

Through the cooperation of the Hill Brothers Packing Company of Topeka, Kansas, adult specimens of *S. vulgaris* were collected and transported to Manhattan, Kansas, in neutral 0.85 per cent saline kept at 37.5° C., the collection and transportation involving no more than 5 hours. Arriving at their destination the worms were washed in neutral (pH 7.1) Locke's solution. One hundred worms were placed in neutral (pH 6.8–7.5) Locke's solution, fresh solution being added every 12 hours. Another hundred worms were placed in an acidified (pH 4.0–5.4) Locke's solution for 12 hours then in neutral Locke's for the remaining time. Each group was kept in an incubator at 37° C. Observations were made at intervals to determine

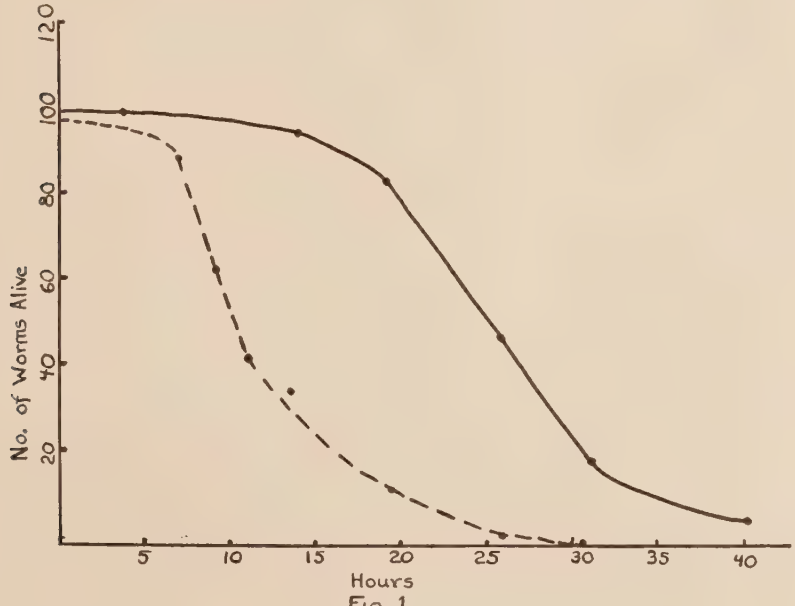

Fig. 1

the numbers of worms alive. Figure 1 shows the results of these observations. The solid line represents the worms kept in neutral Locke's solution, the broken line the worms kept for 12 hours in an acid Locke's solution. It is to be noted that the worms which had spent a period under acid conditions lived a shorter time than the controls in the neutral solution. The death curves of both the control and experimental specimens closely resemble logistic curves.—J. H. Whitlock and E. E. Leasure, *Kansas Agricultural Experiment Station, Contribution No. 72, Department of Veterinary Medicine.*

RESISTANCE OF MYXOSPORIDIAN SPORES TO CONDITIONS OUTSIDE OF THE HOST

Little is known regarding the life of myxosporidian parasites outside the host. Kudo summarizes the situation as follows (Kudo 1921, Tr. Am. Micr. Soc. **40**: 237): ". . . myxosporidian spores have no power of locomotion and have never been seen or made to germinate in water outside of the host." Linton (1891, Bull. U. S. Fish. Comm. **11**: 414) observed spores of *Myxobolus lintoni* Gurley in sea water for ten days. On the 8th to 10th day the contents of the spore rounded up but there was no further evidence of disintegration. After the present study was completed it was found that Thelohan (1894, Bull. Sc. France et Belg. 26: 100–394) had made similar observations on various MYXOBOLIDAE. The spores of the forms he studied were found that Thelohan (1894, Bull. Sc. France et Belg. **26**: 100–394) had made similar Since the period during which the spores are able to retain their structures after they leave the host is an important factor in the study of the transmission of myxosporidia, it was desired to determine the approximate time that spores of *Myxosoma funduli* Kudo, *M. subtecalis* Bond, *Myxobolus bilineatum* Bond, and *Myxidium folium* Bond, would persist outside of the host. These species were all obtained from *Fundulus heteroclitus* (Linn.) collected from Chesapeake Bay.

The studies on the spores were carried out in the following manner: (1) Fresh spores were obtained by teasing apart the infected organ or tissue. (2) Smears were made to determine the stages present and then the remaining spores were sealed in a depression slide (a) in water and (b) in one per cent saline. Three series of each of the above species were studied as follows:

Series 1. Examination of the material each hour for five hours and at the end of that period daily for three days.

Series 2. Examination of the material on alternate days for twelve days, i.e., six examinations.

Series 3. Material examined on the 12th, 15th, 20th and 28th days after it was obtained from the living host.

Examinations by the hanging drop method of Nemezeck (1926, Arch. Protistk. **54**: 137–149) under oil immersion and 12.5× ocular, were supplemented by smears using either Feulgen stain or Heidenhein hematoxylin or both. As criteria of the spore condition, the extrusion of the filament, rounding and vacuolation of the sporoplasm, and the pyknotic degeneration of the nuclei of the sporoplasm were used.

No great difference was found in the length of the period of resistance of spores in saline and water. Spores of *Myxosoma subtecalis* apparently were the least resistant to conditions outside the host. By the 12th day degeneration was general and the majority of the spores showed abnormal structure. Vacuolation of the sporoplasm and pyknosis of nuclei were apparently simultaneous developments. *Myxosoma funduli* did not seem to degenerate as completely as spores of *M. subtecalis*, although the contents of the spore more or less indicated the same status by the 10th to the 12th days. *Myxobolus bilineatum* was the most resistant type of the group when subjected to these experimental conditions. Very little evidence of degeneration was noted by the 28th day, the rounding of the sporoplasm being the only general change. This reaction as compared to other spores studied evidently precedes the actual degeneration or is the first stage in the degeneration of the spore contents.

From the study of the spore contents it is concluded that the life of the spore outside of the host is definitely limited: 12 to 15 days for *Myxosoma subtecalis* and *M. funduli* and approximately 20 days for *Myxidium folium*. An indefinite period was noted in *Myxobolus bilineatum* though some degeneration was observed by the 28th day. There was no actual emergence of the sporoplasm even though the filaments were often extruded. In some spores the amoebulae were noticeably degenerate before extrusion occurred so that this type of extrusion is quite evidently due to some other factor than the activity of the amoebula. No difference was noted between the mono- and the dinucleate spores in their tendency to extrude the filaments.

Thelohan's experiments coincided in general methods and results with those

described above. In several of the species his experiments were carried out for longer periods. Eventual degeneration was found in all. The forms used by Thelohan were mostly of the MYXOBOLIDAE and these, as with *Myxobolus bilineatum*, showed a much longer resistance to conditions outside the host. Thelohan also used the appearance of the sporoplasm, etc., as criteria in determining the condition of the spore.—FRANKLYN F. BOND, *Department of Protozoology, School of Hygiene and Public Health, Johns Hopkins University.*

NOTES ON A GIANT FORM OF THE NEMATODE *NEOAPLECTANA GLASERI*

Occasionally in examining Japanese beetle larvae parasitized with *Neoaplectana glaseri* Steiner, gravid female nematodes of a very large size are encountered. This phenomenon was first observed in field material, and it was thought that some new species of nematode had been found.

In general form and activity, these nematodes differ somewhat from the usual females of this species. The body size is much larger, whereas certain of the internal structures are of normal size, which distorts certain anatomical features. This is particularly noticeable in the esophagus, which, while retaining approximately normal size, occupies a distorted relationship to the intestine and excretory pore. The tail is distinctly more blunt and rounded than usual. Possibly the outstanding difference is the enormously large number of eggs contained in the uterus. These nematodes are inclined to be sluggish, and frequently coil up into a tight helix when disturbed.

That the form is in reality *Neoaplectana glaseri* has been established by infecting beetle larvae with normal nematodes, using larvae and soil known to be free of nematode infection. By culturing the progeny of the resulting giants, normal-appearing offspring were invariably obtained. Attempts to derive a genetic "strain" of large nematodes from such offspring were fruitless.

Well over one hundred offspring of an individual giant female cultured on a standard yeast-agar plate were observed. A number of them were studied for several generations, but no abnormalities of size or fecundity were noticed in any of the descendants.

Later, when a number of these large nemas was again encountered in beetle larvae, five individuals were measured for length, and found to be 9, 8, 8, 6.5, and 5 mm respectively. Steiner (1929, J. Wash. Acad. Sc. 19: 436) gives a length of 4.7 mm for the normal female.

In another case a gravid female giant, length 8 mm, was placed on a culture plate for development. The grub cadaver in which this female was found was carefully triturated, and only one dead male was found associated with the female. This is interesting because it has often been assumed that many or at least several parasites are required to cause the death of the host. Three days after placing this gravid female on the culture plate, all the young had been born and the female was dead. Careful observation over this three-day period showed that no second generation had appeared, and the nematodes on the plate were necessarily the direct offspring of this one giant female. One thousand four hundred and twenty larval nematodes were counted. This is astonishing in view of the fact that Glaser (1932, N. J. Dept. Agric. Circ. 211) reports, that the normal nematode has a total progeny of about 15.

It should be noted that the form is more apt to be encountered in experimental infections where relatively few nematodes are used as inoculum. This has an important bearing on the probable explanation of the origin of this form. Beetle larvae harboring the "giants" are invariably found to be lightly infected, usually less than 10 nemas being present in the host.

This "giant" form has never been produced under conditions where the necessary observations could be made, but the following is a probable explanation of the phenomenon. Bearing in mind, (1) that these "giants" may originate from cultured material whose observed ancestry did not normally produce exceptionally large individuals, (2) that the progeny of "giants" have invariably been normal-sized individ-

uals, (3) that the form is only known to occur in beetle larvae with a very light nematode infection, it is concluded that an abnormal sexual cycle and food supply are responsible. It is presumed that these females are fecundated at a later period than normal (due to the scarcity of males), resulting in an abnormally active somatic development. The food supply within the host would be more adequate, and inhibiting bacteria and metabolic products probably at a low concentration under these conditions.—E. E. McCoy, H. B. Girth and R. W. Glaser, *Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, New Jersey State Department of Agriculture, and the Rockefeller Institute for Medical Research, Princeton, N. J.*

LEUCOCYTOZOON ANATIS WICKWARE, A SYNONYM FOR *L. SIMONDI* MATHIS AND LEGER

Mathis and Leger (1910, Compt. Rend. Soc. Biol. **68**: 119) described *Leucocytozoon simondi* from the blood of a teal duck (*Querquedula crecca*) from Tonkin, China. These authors gave the following description:

> *Macrogametocyte:* oval, 14–15 μ × 4.5–5.5 μ; cytoplasm dark blue, often with many colorless vacuoles of varying size; nucleus red, of irregular shape, usually with a distinct karyosome.
>
> *Microgametocyte:* oval, slightly smaller than female; cytoplasm ashy blue; nucleus pale red, no definite shape.
>
> *Host-cell:* about 48 μ long, with a ribbon-like nucleus along one margin of the parasite and with the cytoplasm extending outward at each pole gradually narrowing to a point.

In a later work (Mathis and Leger, 1911, Recherches Parasitol. et Pathol. Tonkin) these authors quote the above material verbatim from their first publication and also figure a male and a female gametocyte.

Wickware (1915, Parasitology **8**: 17–21), unaware of any previous description of this parasite from ducks, described *"Leucocytozoon anatis"* from ducks in Ottawa, Canada. His description does not differ from that of Mathis and Leger. Wickware states that the length of the host-cell varies from 35 to 60 μ. He figured only macrogametocytes and looked upon the microgametocytes as a variation due to staining characteristics.

O'Roke (1934, Univ. Mich. Sch. Forestry Bull. 4), aware of the figures published by Mathis and Leger (1911) but unaware of the description and name which accompanied these figures, considered the forms he found in many ducks (species not given) to be *Leucocytozoon anatis* Wickware. Although O'Roke's description is more in detail it does not differ from that of Mathis and Leger. He described the microgametocyte as oriented parallel to the elongated nucleus of the attenuated host-cell. The parasite is spindle-shaped, 3.1–4.3 μ × 14.7–18.9 μ; host-cell averages 46.6 μ. The cytoplasm stains a pale blue, is slightly granular, with distinct vacuoles and with pronounced pigment granules. The nucleus is central, oval, 3 × 4 μ, diffuse, pale pink, with no karyosome. The macrogametocyte occurs in the same type of host-cell, is spindle-shaped, 3.2–4.4 μ × 14.5–22 μ; host-cell averages 55.4 μ. The cytoplasm stains dark blue, is granular, with distinct vacuoles and numerous pigment granules. The nucleus is central, spherical, 3.1 μ in diameter, and contains a distinct karyosome. O'Roke bases his dimensions on 100 measured forms.

The present author has observed *Leucocytozoon* in the blood of 3 species of Anatidae at the Austin Ornithological Research Station on Cape Cod. During the summer of 1936 these parasites were observed in the blood of several common black ducks (*Anas rubripes tristis*) (Herman, 1938, Tr. Am. Micr. Soc., **57**: 132), and was considered to be *Leucocytozoon anatis*. During the summer of 1937 *Leucocytozoon* was observed in the blood of a red-breasted merganser (*Mergus serrator*) and a blue-winged teal (*Querquedula discors*). In studying the last form, the possible synonomy of *Leucocytozoon anatis* and *L. simondi* developed. The parasites of the three species of ducks examined on Cape Cod seem to be morphologically the same. The sizes of the parasites and of the parasitized host-cells are considered to be poor

differential characters in view of the wide variations within a single host, and the average size of only 100 forms from a single bird may not be a statistically adequate basis for diagnosis. Coatney and Roudabush (1937, Am. Mid. Nat. **18**: 1005–1030) point out that "most of the workers concerned with the leucocytozoa of ducks have failed to identify the species, or even the genus of ducks with which they worked. Because of this nothing definite can be said about the host-specificity of this form nor can a definite decision be arrived at concerning the synonomy." If there should prove to be a host-parasite specificity of these leucocytozoa, in view of their similar morphology *L. anatis* can, at best, be considered only a subspecies of *L. simondi* and not a distinct species.

Coatney and Roudabush have suggested that if *Leucocytozoon anatis* Wickware and *L. simondi* Mathis and Leger are the same species, then the latter name is the correct one for the parasite. The present author has presented evidence indicating that these two parasites are morphologically the same and that therefore *Leucocytozoon simondi* is the correct name and *L. anatis* should be reduced to synonomy.—Carlton M. Herman, *Department of Protozoology, School of Hygiene and Public Health, Johns Hopkins University.*

THE STAINING OF *TRICHOMONAS FOETUS* RIEDMÜLLER, WITH WRIGHT'S BLOOD STAIN

The time consumed and the difficulty experienced in making satisfactory stained preparations of *Trichomonas foetus* with hematoxylin, hemalum, and Giemsa stains led to experiments with Wright's blood stain. A satisfactory procedure was developed which is as follows:

1. Place material to be stained on a clean glass slide.
2. Fix with osmic acid fumes.
3. Smears may be dried in air but this is unnecessary.
4. Cover smear with 5 drops of Wright's stain.
5. Add 15 drops of Sørenson's buffered solution with a pH between 7.0 and 7.6.
6. Allow to stand 2 minutes.
7. Wash with Sørenson's buffered solution.
8. Dry in air.

One drop of culture medium containing *Trichomonas foetus* is placed on a clean glass slide and spread over an area about 2 cm in diameter. Fixation of the flagellates by osmic acid fumes is accomplished by inverting over the material to be stained a 5 ml beaker, in the bottom of which has been placed a piece of folded filter paper (Whatman 5.5 cm, no. 5). Immediately before use, 1 or 2 drops of a 2 per cent osmic acid solution in a 1 per cent chromic acid solution are placed on the filter paper. Trichomonads should be exposed to the osmic acid fumes until microscopic examination shows no motility among the organisms; 10–30 seconds is usually sufficient. Despite the fact that osmic acid is said to undergo rapid reduction in the presence of the least amount of organic matter, under conditions of this procedure its efficiency as a fixative does not seem to be lessened by contact with filter paper at the time of use.

It is unnecessary to dry smears before staining them; but many organisms are washed off the slide by applying stain to a wet smear. The organisms are not distorted and their staining potentialities are not lessened by the drying process. It therefore seems advisable to dry smears before staining them.

Five drops of commercially prepared Wright's blood stain are placed on the smear. To this is added, immediately, 15 drops of Sørenson's buffered solution, with a pH between 7.0 and 7.6. The solution is mixed by tilting the slide gently 2 or 3 times. Diluted stain is allowed to stand about 2 minutes. It is then "floated off" and the slide washed thoroughly with the Sørenson's solution previously used as the stain diluent. The preparation is dried in air and is ready for microscopic examination.

Trichomonas foetus stains according to the characteristic Romanowsky staining reactions. The anterior flagella and those bordering the undulating membrane be-

come pink, the cytoplasm light blue, the posterior part of the axostyle pink and that portion extending through the cytoplasm blue, and the blepharoplasts, chromatin ring around the posterior portion of the axostyle, endoaxostylar granules and nucleus, a dark purple. The cytostome appears as a clear area at the anterior extremity of the body of the organism.

The above method of fixation requires a minimum amount of time and an extremely small quantity of osmic acid, and allows the material to be stained to remain within a limited small area, thus facilitating rapid examination of the slide.

The entire process requires about 15 minutes; results are much more uniform than in those methods involving destaining; and the staining reactions are apparently not altered by slight changes in the hydrogen ion concentration of the culture medium in which the organisms are suspended.

The writer is indebted to Dr. Justin Andrews for the suggestion of the possibility of using Wright's stain on *Trichomonas foetus*.—HELEN M. STEWART, *Department of Protozoology, School of Hygiene and Public Health, Johns Hopkins University.*

SCHISTOSOME DERMATITIS IN DOGS

During the summer of 1937 a vacation resident of Topinabee, Michigan, brought a dog to the University of Michigan Biological Station for observation of a rash along the lower part of its body. From conversation with the owner it was learned that occasionally after coming out of the water the dog would scratch its abdomen, sometimes until bloody areas appeared.

On the possibility that the dog's itching was caused by infection with a schis-

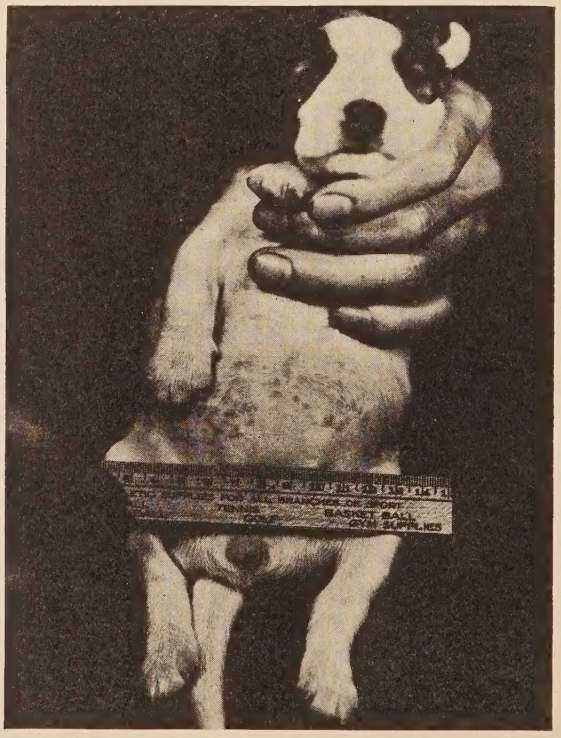

FIG. 1. Lesions of schistosome dermatitis 12 hours after exposure.

tosome cercaria, a 14-day old mongrel was located upon which to test the hypothesis. This dog had very short hair on the abdomen, and showed no red spots of any character in the skin. Cercariae, 12 hours old, of *Schistosomatium douthitti,* were concentrated from milk bottles containing infected *Lymnaea stagnalis appressa* Say. With this cercarial suspension infection was attempted by wetting the abdomen of the dog for twenty minutes. Then the moistened area was allowed to dry. Within ¾ hour after first applying cercariae distinct red spots had developed until over one hundred were countable. Their prominence increased until some single lesions were perhaps 2 mm in diameter. Others were so close together they coalesced and could be photographed (Fig. 1). In this case no itching or irritation was noted, nor were any constitutional effects observed. Two days afterwards the rash had largely disappeared and by the evening of the fourth day after exposure, entirely so.

It is well known that this and other non-human schistosome cercaria produce a dermatitis in human beings (Cort and Talbot, 1936, Am. J. Hyg. **23**: 349–396). This case suggests that schistosome dermatitis in man and dogs is possibly similar, the severity of the reaction probably depending upon the number of cercaria penetrating and individual susceptibility.—E. C. Herber, *University of Michigan Biological Station.*

NATURAL ELIMINATION OF *GIARDIA MURIS* FROM RATS

Do rats lose their infections with the flagellate, *Giardia muris,* when reinfection is prevented? Forty rats were found by fecal-pellet examination to be infected with *Giardia muris.* Twenty of them were placed in a large cage with a screen bottom through which fecal pellets evacuated by the rats would pass, carrying with them any giardia cysts that might otherwise be ingested and reinfect the animals. The remaining twenty rats were kept in a large cage on shavings. After a period of five months, all the rats were killed and the small intestine examined throughout for giardias. The twenty rats that had lived in the cage with a screen bottom were all negative. Nineteen of the other group were positive. The approximate total numbers of giardias obtained from the entire small intestine of seventeen of these rats were as follows: 150,000; 150,000; 300,000; 300,000; 600,000; 1,050,000; 1,050,000; 1,800,000; 2,250,000; 2,250,000; 2,250,000; 3,600,000; 4,050,000; 5,100,000; 10,500,000; 10,800,000; 12,600,000. In our trichomonad-free colony of rats, kept in cages on shavings, the young become infected with giardias and maintain their infections as long as we have kept them under observation, a period of many months. These results indicate that rats may lose their infections with *Giardia muris* if reinfection is prevented and that persistent infections are due to reinfection.—Robert Hegner and Lydia Eskridge, *Department of Protozoology, School of Hygiene and Public Health, Johns Hopkins University.*

AMERICAN SOCIETY OF PARASITOLOGISTS

PRELIMINARY ANNOUNCEMENT OF THE FOURTEENTH ANNUAL MEETING

WEDNESDAY, THURSDAY AND FRIDAY, DECEMBER 28–30, 1938

RICHMOND, VIRGINIA

Pursuant to action taken at the last annual meeting in Indianapolis, the American Society of Parasitologists will convene for a three-day program in conjunction with the meeting of the American Association for the Advancement of Science in Richmond, Virginia. Hotel Richmond will be headquarters for the Society and the program sessions will be held in McGuire Hall at the Medical College of Virginia.

The program will be arranged in accordance with the custom of recent years with the first and third days devoted to the reading of contributed papers. The presidential address by Dr. F. C. Bishopp on the subject "Some problems in medical and veterinary entomology" will be delivered at the conclusion of the morning session of the second day. The annual luncheon and business meeting will follow the presidential address, and the demonstration program and tea will be held in the afternoon. The demonstration-tea is planned to serve both as a scientific program and as a social function, and to supply the opportunity for informal discussion that is not possible at the regular scientific sessions.

The call for papers has already been sent to members of the Society. In order that the abstracts be published and mailed to the membership before the meetings, it is necessary that they be received in the office of the secretary not later than November 4, 1938. Members are hereby reminded of this date, and are requested to be prompt in the submission of papers for the program.

O. R. McCOY, *Secretary*